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VOL. 88 JULY–AUGUST 1979 No 1–2

EDITOR. C.-A. HAMBERGER STOCKHOLM

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Conferences and Meetings

1979 July 22–27 The Fifth British Academic Conference in Otolaryngology to be held at the University of Birmingham, UK. Addr. V Hammond FRCS 55 Harley Street, London W 1 England.

1979 Sept. 6–8 International Symposium on Sensorineural Hearing Loss, Vertigo and Tinnitus to be held at the University of Minnesota, Minneapolis, USA. Addr Ms Ruth McIntyre, Continuing Medical Education, Box 293 Mayo Memorial Building, University of Minnesota, 420 Delaware Street S.E., Minneapolis, MN 55455 USA.

1979 Sept. 26–28 IVth International Symposium on Acoustic Impedance Measurements to be held in Lisbon, Portugal. Addr Dr Paulo Noronha Pizarro Clinica Fono-Audiologica, Rua Conde Redondo 119-3 Lisboa, Portugal.

1979 Oct. 5–6 XVI Course on Temporal Bone Dissection and Ear Surgery will be held in Red Cross Hospital, Barcelona, Spain. Addr Secretary of the Courses, Muntaner 366-3° Barcelona-22, Spain.

1979 Oct. 18–20 The Italian Congress of Maxillofacial Surgery will be held in Saint Vincent (Aosta Valley) Addr F Mela, Department of Maxillo-Facial Surgery Clinica Odontostomatologica dell'Università, Corso Polonia, 2, 10126 Torino Italy

1979 Nov 18–20 Semi-Annual VII Nerve Surgical Dissection Course to be held in Elmsford, New York. Addr Ms P Tamkin, c/o Dr P Guibor 630 Park Ave., New York, NY 10021 USA.

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MORPHOLOGICAL ALTERATION OF THE STRIA VASCULARIS AFTER ADMINISTRATION OF THE DIURETIC BUMETANIDE

Peter A Santi and Arndt J Duvall III

From the University of Minnesota Department of Otolaryngology Medical Research East Minneapolis Minnesota, USA

(Received October 3 1978)

Abstract Chinchillas were given either a single injection of the diuretic bumetanide (18 mg/kg body weight) or saline-sodium hydroxide and sacrificed at 10 min 1 hr and 24 hr after the injection *Slight stria edema was present* at 10 min, marked edema at 1 hr and no edema 24 h after bumetanide. The edema began in the first cochlear turn at 10 min and spread to the second turn by 1 hr Along with edema, marginal cell bulging, potential capillary constriction and the formation of marginal cell membranous structures occurred after bumetanide treatment.

Bumetanide is an aminobenzoic acid derivative which is related to furosemide in chemical structure and is classified as a loop inhibiting" diuretic Bumetanide s primary renal action is inhibition of sodium and water reabsorption in the loop of Henle (Hropot & Muschaweck 1975)

Brown has investigated bumetanide ototoxicity in the cat and the guinea pig by measurements of cochlear microphonics N1 and endocochlear potentials (Brown 1974 1975 1976*a* *b*) The ototoxicity of bumetanide was found to be less than that of furosemide and Brown s data suggest that bumetanide s primary cochlear effect was depression of the endocochlear potential Since it is generally recognized that the source of the endocochlear potential is the stria vascularis it is this cochlear tissue that is most likely to be affected by bumetanide

Morphological changes in the stria vascularis after other loop inhibiting" diuretics such as ethacrynic acid and furosemide have been previously described (e g. Quick & Duvall 1970 Bosher et al 1973 *Brummett* et al. 1977) Alterations in stria morphology that have been described include edema, cyst like areas of edema near Reissner's membrane marginal cell cytoplasmic changes intermediate cell degeneration and generalized cell shrinkage Similar stria alterations would be expected after bumetanide however the effect of the drug on stria morphology is not known.

Due to the potential clinical significance of bumetanide and its use as a tool to develop quantitative techniques for the assessment of stria "pathology" the present investigation on the effect of bumetanide on the chinchilla stria vascularis was conducted

METHODS

Fifty-four chincillas were divided into eight groups There were three bumetanide-treated groups with *sacrifice time of 10* min 1 hr and 24 hr and a non-treated normal" control *group* each containing 7 animals The bumetanide control groups containing 13 4 and 5 animals per group received a saline-sodium hydroxide injection and were sacrificed at 10 min 1 hr and 24 h. In addition, 4 animals – *sacrificed 1 hr* after bumetanide and 2 sacri*ficed 1 hr* after saline–sodium hydroxide treatment, were used for examination of the stria surface The animals were anesthetized with 60 mg/kg/body weight sodium pentabarbitol (*Diabutal*) and received a single intravenous injection of bumetanide or saline-*sodium hy*droxide To dissolve bumetanide, the powder was placed *in 0.2 ml*/kg/body weight 1 N so-

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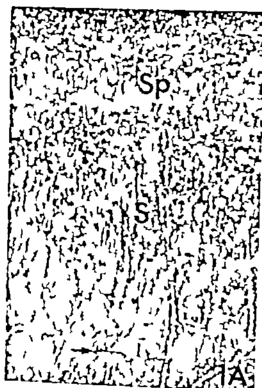
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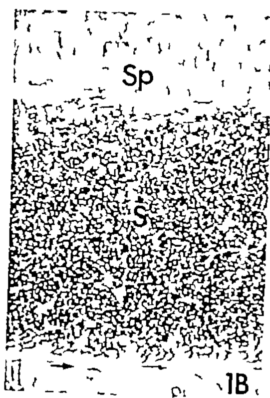
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dium hydroxide and then added to 0 7 ml of saline The solution was injected over a period of 15 sec into the right jugular vein

After the specified time period the animals were decapitated and the cochleae were quickly (less than 2 min) removed from the animal and perfused through the round and oval windows with either 1–2% glutaraldehyde or 2% osmium tetroxide in phosphate buffer at pH 7 3 for light and electron microscopy

For surface examination of the stria glutaraldehyde fixed cochleae were decalcified for 5 days with 5% EDTA After decalcification the cochleae were cut mid modiolar and the complete stria in half turn segments were removed and mounted on slides in glycerol

For light microscopy Epon-embedded whole scala media cross sections of each of the three cochlear turns were cut at 1 μm thickness and stained with toluidine blue Three measurements of stria thickness (the distance from the apex of the marginal cells to the base of the basal cells) were obtained along the stria s width (which extends from the attachment of Reissner s membrane to the spiral prominence) These thickness measurements were made with an eyepiece micrometer at intervals of 1 7 μm at a light microscope magnification of ×600 Approximately the same quadrant from each of the three cochlear turns was measured to give a mean stria thickness for each turn of every cochlea. The turn means were pooled within each group

For electron microscopy the Epon-embedded blocks were sectioned at 60 nm placed on grids and stained with uranyl acetate and lead citrate The grids were examined and photographed in either a Zeiss 10 or a JEOL 100S transmission electron microscope

RESULTS

Light microscope observations

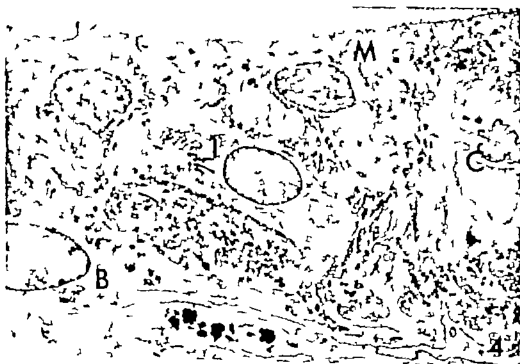
The most striking morphological change in the stria vascularis after bumetanide was edema Observations from 1 μm cross sections of the stria revealed slight edema at 10 min marked edema at 1 hr and no edema 24 hr after bumetanide administration Fluid accumulation within the stria increased its overall thickness and was used as a quantitative measure of stria pathology It was found that some bumetanide-treated animals were more severely affected than others however no consistent morphological changes were observed in control animals

Surface preparations of the stria vascularis were obtained from normal 1 hr bumetanide and 1 hr control chinchillas In normal and 1 hr control animals the endolymphatic surface of the stria was smooth and undifferentiated Stria capillaries and pigment could be observed but marginal cell borders were indistinct (Fig 1 A) In contrast surface preparations of 1 hr bumetanide treated strias revealed diffusely distributed edema which appeared more severe in the basal rather than apical turns The edematous regions of the stria could be recognized due to bulging of the marginal cells into the endolymphatic space (Fig 1 B) In addition stria capillaries (1 hr after bumetanide) appeared to have narrowed lumina containing fewer erythrocytes than capillaries in 1 hr control and normal animals

Cross sections through some of the strias in the 1 hr bumetanide animals revealed increased

Fig 1 A A light photomicrograph of an unstained surface preparation of the stria vascularis in a 1 hr control (saline–sodium hydroxide) injected animal The arrow indicates the attachment of *Reissner s membrane SP Spiral* prominence *S* stria vascularis The endolymphatic surface of the stria appears smooth and undifferentiated and marginal cell borders were not clearly defined. ×700.
Fig 1 B A light photomicrograph of an unstained surface preparation of the stria 1 hr after bumetanide administration Note the attachment of Reissner s membrane (*arrow*) the spiral prominence (*SP*) and the edematous state of the stria (*S*) with bulging of the marginal cells into the scala media ×700

Fig 2 A cross section of a toluidine blue stained 1 μm Epon section of the stria in 1 hr bumetanide animal Note the spiral ligament (*SL*) and the cystic area (*C*) of increased stria edema near Reissner membrane (*arrow*) ×1 700



A transmission electron micrograph of cross section through the stria in I he control animal. Stria morphology appears normal the marginal (M) form flat endolymphatic surface intermediate (I) and basal (B) are present, and stria capillaries (C) are filled with erythrocytes 4500

Fig. 5. Ten minutes after bumetanide administration the stria is slightly edematous. Expanded intercellular spaces (arrows) most commonly occur near capillaries and intermediate cell 4900

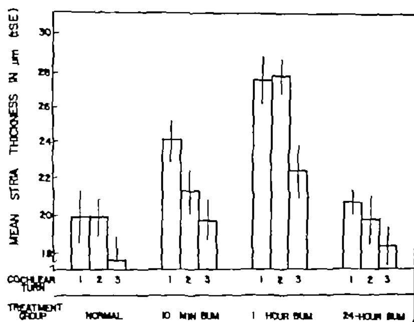


Fig 3 Mean stria thickness (±S.E.) measurements are plotted for each treatment and the three cochlear turns. After bumetanide stria edema begins at 10 min in the first turn and spreads to include the second turn at 1 hr After 24 hrs stria thickness has returned to normal in all cochlear turns.

edema near the attachment of Reissner s membrane (Fig 2) These regions were approximately twice as thick as adjacent areas of the stria and usually involved only the upper 20% (near Reissner s membrane) of the stria s width

Measurements of mean stria thickness were obtained from the 1 µm cross sections of osmium fixed tissues The results of these measurements for the three cochlear turns of each of the four groups (normal 10 min 1 hr and 24 hr bumetanide) are shown in Fig 3 Statistical tests (analyses of variance and subsequent *t* tests with significance levels corrected for multiple comparisons) were conducted on the quantitative data There were no significant differences between normal and control animals thus only normals were compared with bumetanide groups with respect to sacrifice time and cochlear turn A statistically significant difference was found between sacrifice time and cochlear turn ($p<0.01$) however there was no significant statistical interaction between these factors The only significant differences among turns were between turns 1 and 3 in the 1 hr bumetanide group ($p<0.002$) and between 1 and 3 in the 10 min bumetanide group ($p<0.001$) The only significant differences among treatment groups were between the normals and 1 hr group ($p<0.001$) and 1 hr and 24 hr ($p<0.001$) bumetanide animals

Transmission electron microscope observations

Normal chinchilla stria morphology in a 1 hr control animal is shown in Fig 4 The marginal cells form a relatively flat endolymphatic surface Lightly stained intermediate cells and two layers of basal cells are also present Stria capillaries appeared normal in size and were usually packed with erythrocytes

Ten minutes after bumetanide administration only slight edema was present within the stria (Fig 5) These fluid filled intercellular spaces commonly occurred between stria capillaries marginal and intermediate cells No consistent organelle changes were found in the stria cells 10 min after bumetanide The surface of the stria showed some slight bulging of the marginal cells into the endolymphatic space Stria capillaries did not show any consistent changes 10 min after bumetanide

One hour following bumetanide large areas of intercellular spacing (edema) were evident among stria cells and capillaries (Fig 6) For the most part these spaces were electron-lucent but some sparsely distributed flocculent material could be observed near the cell



Fig. 4. A transmission electron micrograph of cross section through the stria in the control animal. Stria morphology appears normal: the marginal cells (M) form flat endolymphatic surface; intermediate (I) and basal cells (B) are present and stria capillaries (C) are filled with erythrocytes. ×4700.

Fig. 5. Ten minutes after bumetanide administration the stria is slightly edematous. Expanded intercellular spaces (arrows) most commonly occur near capillaries and intermediate cells. ×4900.

membranes The endolymphatic surface of the stria was no longer smooth but scalloped due to bulging of the marginal cells into the endolymphatic space The marginal cells were separated by the edematous fluid up to their apical tight junctions which appeared strained (Fig 7) but apparently intact Marginal cell processes were elongated but retained their orientation towards the basal cell layer

An unusual structure was observed in the infranuclear zone of the marginal cells in the 1 hr bumetanide animals However these structures were also observed less frequently in the 10 min and 24 hr bumetanide treated animals in controls and even in apparently normal animals The structures consist of a discrete somewhat spherically shaped network of plasmalemma infoldings or vesicles and tubules of the marginal cell (Fig 8 A B C) In glutaraldehyde fixed strias plasmalemma infoldings were clearly evident with some infoldings having communication with the intercellular space (Fig 8B) In general these structures appeared more frequently and were more complex in the 1 hr than in the 10 min bumetanide animals In glutaraldehyde fixed strias showing slight or no edema the space between the plasmalemma infoldings maintained an approximately normal intercellular distance of 10–20 nm In animals with marked edema this intermembranous space was larger and more variable In stria tissue fixed only with osmium tetroxide the plasmalemma infoldings were less clearly defined and this structure appeared to be primarily composed of electron lucent tubules and vesicles or vesicotubules (Fig 8C)

One hour after bumetanide the intermediate cells appeared to have fewer processes (Fig 6) but none were found that could be classified as degenerating Nevertheless some broken intermediate and basal cell plasma membranes were observed in strias with marked edema When compared with controls and normals some stria capillaries appeared constricted 1 hr after bumetanide with their endothelial nuclei having a crenated appearance Capillary lumina were narrower and not packed with erythrocytes as was commonly observed in normal and control animals In addition some endothelial cells showed mitochondrial cristae damage and protrusions of the endothelial cell cytoplasm into the vessel lumen (Fig 8D) Stria basal cells were the most stable cell type after bumetanide treatment as no consistent morphological changes in basal cell morphology were observed other than occasional broken plasmalemma membranes The tight junctions of the basal cells appeared unaffected after bumetanide

Twenty four hours after bumetanide administration no stria edema was present though stria morphology was not completely normal The differentiation between stria cell processes was less distinct as the cytoplasm of the marginal cells was less osmiophilic and appeared coarse (Fig 9) Stria capillaries appeared to have returned to normal as they were packed with erythrocytes and did not appear constricted

DISCUSSION

Surface examination of the stria vascularis 1 hr after bumetanide treatment revealed diffusely distributed edema which appeared more severe in the basal portion of the cochlea Stria edema was characterized by increased stria thickness and bulging of the marginal cells into the endolympatic space Using edematous changes in stria thickness as a quantitative indicator of pathology it was shown that

Fig 6 One hour after bumetanide administration the endolymphatic surface of the stria is scalloped due to bulging of the marginal cells Stria edema is pronounced and marginal cell membrane structures (×) are found in the infranuclear zone of some marginal cells The intermediate cells appear to have fewer processes but the basal cells are relatively unaffected after bumetanide ×3 900

Fig 7 One hour after bumetanide the marginal cells *appear to be compressed along their* latero-basal borders and bulge into the scala media. Their apical cell junctions are strained but apparently intact (*arrow*) A marginal cell membranous structure (×) is also present 7 800


X
7

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9 The stria 4 hrs after bumetanide treatment. Stria capillaries are filled with erythrocytes and appear relatively normal. Marginal cell cytoplasm was less osmiophilic and appeared coarse. ×4300

) there were no statistically significant differences in stria thickness between normal (untreated) chinchillas and the control (saline-sodium hydroxide treated) animals at any sacrifice time (2) among the treatment conditions there were significant differences between normal and 1 hr and between 1 hr and 4 hr bumetanide treated animals and (3) stria thickness was greater in the basal rather than apical portion of the cochlea at 10 min and 1 hr after bumetanide

Most previous investigators have reported that the basal rather than apical portion of the cochlea is more severely affected by ethacrynic acid However it is not clear from their data whether the cochlear base is more susceptible to the diuretic or whether the effect of the diuretic begins in the base of the cochlea and travels to the apex By measuring edema in the three cochlear turns at different sacrifice times the present results indicate that bumetanide s affect begins in the 1st turn at 10 min and extends to the 2nd and 3rd turns by 1 hr

At 10 min after bumetanide treatment edema most commonly occurred around blood vessels and intermediate cells This observation suggests that the edematous fluid may initially arise from the stria capillaries At 1 hr after bumetanide treatment, when edema was greatest, stria capillaries appeared constricted and marginal cells bulged into the endolymphatic space Thus, constricted capillaries and marginal cell bulging may be the passive result of increased hydrostatic pressure within the stria compartment. It should be noted that vessel contents (erythrocytes) and diameter appear to be related to the fixation method When perilymphatic perfusion of the fixative is performed after decapitation normal stria capil-

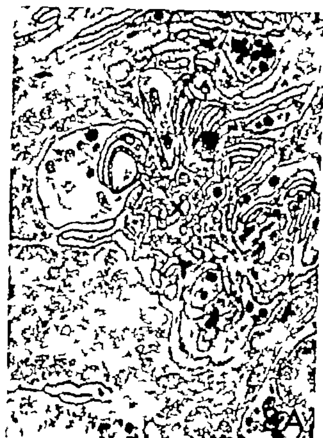








Fig 8A A marginal cell membranous structure (×) showing membranous infoldings of the marginal cell in a 10 mm bumetanide animal ×13 400

Fig 8B Marginal cell membranous infoldings showing communication with the intercellular space (*arrow*) in a glutaraldehyde-fixed stria 1 hr after bumetanide ×16 000

Fig 8C Marginal cell membranous structure showing vesicotubule (*Vt*) formation in an osmium-fixed stria 1 hr after bumetanide ×15 200

Fig 8D A constricted stria capillary showing endothelial protrusions (*P*) into the vessel lumen and mitochondria with cristae damage ×14 600.

cellular metabolism—the formation of a discrete membranous structure in the infranuclear zone of the marginal cell When osmium tetroxide fixation is used the structure appears to be primarily composed of vesicotubules which is probably due to fixation artifact. After glutaraldehyde fixation the structure appears as plasmalemma infoldings of the marginal cell some of which communicate with the intercellular space Among the bumetanide treated animals this membranous structure occurred most frequently and with a more complex morphology in animals sacrificed 1 hr after bumetanide We have observed this structure in other bumetanide treatment groups after acoustic trauma, in animals rendered experimentally diabetic and in bumetanide control and apparently normal animals. The morphology of this structure suggests that its function is related to fluid transport. Numerous other examples of cells with similar morphological specialization for transport of fluids have been described (for a review see Berridge & Oschman 1977) These fluid transporting cells have been reported to change morphology with different fixatives (Sedar 1962 Lillibridge 1968) and with changes in metabolic states Both elaboration of the internal tubular structure or increased infolding and intercellular spacing has been described after these cells have been induced to increased fluid transport (Kessel & Beams 1962 Shirai & Utida, 1970 Kaye et al 1966 Tormey & Diamond 1967)

It has been suggested that stria marginal cells are the primary site for fluid transport and production of the positive endocochlear potential. Their morphology is similar to other fluid transporting cells and it seems likely that these plasmalemma infoldings found in chinchillas, are not degenerative but represent increased marginal cell fluid transport which may be related to the diuretic treatment

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Chinchillas erhielten entweder eine einmalige Injektion von dem Diuretikum Bumetanide (18 mg/kg Körpergewicht) oder ein gleiches Volumen physiologische Kochsalzlösung Die Tiere wurden getötet 10 min, wie 1 h und 24 h nach der Injektion. I den Bumetanide Tieren wurde leichtes Ödem im Stria, schweres Ödem nach 1 h und kein Ödem nach 24 h gefunden. Das Ödem begann nach 10 min in der ersten Schneckenwindung und erreichte die zweite Schneckenwindung in der ersten Stunde. Zusätzlich zum Ödem wurden nach der Bumetanide Verabreichung Marginalzell-Quellung mit Bildung von Membranstrukturen und Andeutung von Kapillarkonstriktion beobachtet.

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laries are usually filled with erythrocytes while 1 hr after bumetanide treatment stria capillaries appear constricted When perilymphatic perfusion of the fixation is performed *in vivo* normal stria capillaries do not appear to fill with erythrocytes nor does it seem that stria capillaries are constricted 1 hr after bumetanide treatment Further quantitative examination of stria capillaries are necessary to resolve this discrepancy An additional finding which may be related to the vascular supply to the stria was the cyst like region of increased stria edema near Reissner's membrane Similar cyst like areas of edema in the same region have also been reported in the cat after ethacrynic acid treatment (Silverstein & Yules 1971 Silverstein & Begin 1974) In the guinea pig (Hawkins et al 1972) and chinchilla branches of the radiating arterioles enter the stria near the attachment of Reissner s membrane These vessels are the first to enter the stria and presumably contain the highest concentration of bumetanide and thus may be responsible for the localized increase in edema We are currently attempting to describe quantitatively any potential changes in vascular permeability or capillary diameter after bumetanide treatment in order to resolve this point Nevertheless there are at least three other potential sources for the edematous fluid intercellular perilymph and endolymph

Brummett et al (1977) and Matz (1976) have described stria cell shrinkage after ethacrynic acid Brummett et al have postulated that movement of intracellular fluids (containing ions) into the interstitial space contributes to edema formation After ethacrynic acid or bumetanide stria cytoarchitecture drastically changes large edematous spaces predominate the marginal cells bulge at their apex and elongate at their base and the intermediate cells appear to have fewer processes However without quantitative data it is difficult due to the drastic change in stria morphology to determine whether any stria cell volume changes have occurred and contributed to the formation of stria edema We are presently investigating any potential stria cell volume changes after bumetanide treatment using morphometric and stereological techniques

Basal cells appeared to be relatively unaffected after bumetanide and other diuretics Basal cells usually form several cell layers which are tightly joined by intercellular junctions (Reale et al 1975) These junctions seal the compartment of the stria from the perilymph of the spiral ligament. Since the junctions are extensive and appear to remain intact after diuretic treatment, it is unlikely that perilymph could enter the stria from the spiral ligament and contribute to the edematous swelling

One hour after administration of bumetanide marked stria edema was present The marginal cells bulged into the endolympatic space and their apical junctions were strained but apparently intact In the guinea pig Brummett et al (1977) have also reported time dependent marginal cell bulging after ethacrynic acid Rawlins et al (1975) have reported widening of kidney tubule cell tight junctions after perfusing the tubule lumen with mannitol At present it is not known whether bumetanide or any other diuretic is capable of widening marginal cell tight junctions and thus providing a paracellular route of fluid transport between the stria and the scala media.

Basal infoldings of the stria marginal cells have been described on the electron microscopic level in the human and several animal species These membranous folds form numerous basal cell processes (packed with mitochondria) which apparently serve to increase the surface area of the cell This description also applies to the chinchilla marginal cell as well Rodriguez Echandia & Burgos (1965) and Hinojosa & Rodrigues-Echandia (1966) have noted that similar morphological specializations commonly occur in other cell types that are engaged in active fluid transport e g renal tubule cells nasal salt rectal salt and salivary gland cells

In the chinchilla we have observed under certain circumstances—presumably related to

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PHYSIOLOGICAL ACTIVATION OF THE STAPEDIUS MUSCLE IN *GALLUS GALLUS*

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Abstract The function of the avian middle ear muscle was investigated in the chicken *Gallus gallus* (domesticus) The avian species offers excellent conditions for study of middle ear muscle function since it possesses single middle ear muscle the stapedius, which is located extracranially Electromyograms (EMG), measurements of impedance change and volume change in the middle ear cavity were used to assess the muscle activity The results showed that the middle ear muscle of Gallus does not exhibit an acoustic reflex. However the stapedius is regularly activated during the animal' own vocalization. Measurements of the EMG and volume change showed the stapedial activity to increase systematically with increases in the vocal sound level The use of volume change as a measure of stapedius function was found to be highly suitable in the present experiments in that it allows for measurements of the magnitude of the stapedius contraction without altering the intact physiological state of the middle ear and is insensitive t the ambient noise and vocal sounds, that hamper the impedance technique

The middle ear of Aves has received little experimental attention in comparison with Mammals It differs from that of mammalians in several significant respects (*a*) The avian middle ear has a large circular convex tympanic membrane which bulges outward at the point where it attaches the ossicle (*b*) It has a single plunger-shaped ossicle the columella, which is connected to a small cartilaginous extra columella. (*c*) It also has an intracranial communication via a continuous air-filled space (Wada, 1974) and (*d*) the ear of birds has a single middle ear muscle the stapedius which is situated mainly outside the middle ear cavity and is innervated by a branch of the facial nerve (Smith, 1904 Pohlman 1971) The avian middle ear is thus largely simpler than the mammalian middle ear Furthermore, the location of the stapedius muscle outside of the middle ear cavity allows for physiological studies under intact middle ear conditions Information regarding the physiology of the stapedius of Aves is however very sparse and partly contradictory

Wada (1924) examined the function of the stapedius in pigeons during acoustic stimulation but failed to demonstrate any acoustic reflex activity Golubeva (1972) on the other hand found regular acoustic reflexes in owls Oeckinghaus & Schwartzkopff (1975) found that electrical stimulation of the stapedius of starlings caused an attenuation of pure tone cochlear microphonics However there appears to be no information regarding the muscle s function during vocalization

In the present study several experiments were performed on the chicken *Gallus gallus* in order to answer certain basic questions about the physiology of the avian stapedius (*a*) whether there is an acoustic reflex activation of the stapedius (*b*) whether the stapedius is activated during vocalization *Gallus* was considered a suitable preparation for these studies because of its capacity for middle (sonic) frequency hearing and spontaneous vocalization and its availability size and growth control.

METHODS

The experiments were performed in 12 chickens 1–12 weeks old Surgical preparations were performed under Nembutal anesthesia (60 mg/kg body weight) All measurements were made after at least hours which is ade

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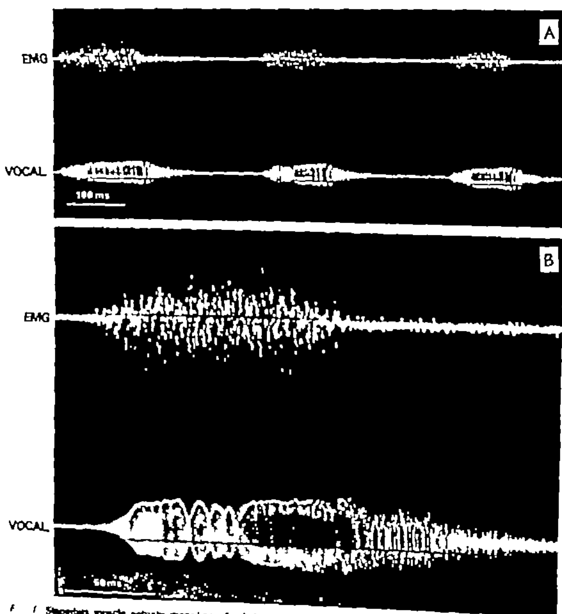


Fig. 1. Stapedius muscle activity recording of lightly anesthetized chicken during chirping. *Upper trace*: Electromyogram obtained with bipolar stainless steel electrode. *Lower trace*: vocal sound recorded 20 cm in front of the animal. *A*: slow time base. *B*: rapid time base.

served at higher levels were always associated with head movements.

B. Stapedius activity during the animal's own vocalization

The impedance changes could not be used as a measure of stapedius muscle activity during vocalization since the chirp sound itself distorted the probe signal. However, we obtained regular evidence of stapedius muscle activity both as EMG and volume changes during vocalization. In addition, eardrum movements and movements of the stapedius muscle belly could be observed under the dissecting microscope during vocalization.

Fig. 1 shows EMG recordings obtained by

quate time for recovery of middle ear reflex function (Borg & Møller 1975) After the recovery period the animals moved and chirped spontaneously

The activity of the stapedius muscle was assessed in three ways Firstly acoustic impedance change was recorded in both ears simultaneously with a technique developed by Møller (1961) In three of the experiments the outer ear canal was removed and a tube glued directly to the outer rim of the tympanic membrane In four other experiments the rubber tube was inserted into the intact ear canal close to the ear drum and sealed with tissue glue The impedance change was measured at 800 Hz with a tone level of 71 or 77 dB SPL (sound pressure level re 20 μPa)

Secondly electromyograms (EMG) were obtained from the stapedius by a bipolar platinum electrode (0 1 mm Ø) or a bipolar stainless steel electrode isolated except at the tip The bio-electric signals were amplified (Grass 7P3 amplifier) filtered (80–8 000 Hz) and recorded on a Grass pen writer (79D) and on a Revox 35A magnetic tape recorder for later processing

Thirdly the volume changes in the middle ear cavity were measured by a 12-gauge cannula which was glued to a hole drilled in the temporal bone near the dorsal rim of the tympanic ring Volume changes in the whole middle ear system were measured by a Grass PT5A volumetric pressure transducer This device measures volume changes without introducing significant pressure changes into the system Dynamic pressure changes as small as 0 002 mm Hg may be observed with this transducer Volume changes were recorded on a Grass 79D system and a Tandberg FM magnetic tape-recorder

The volume measurement technique was adapted for the present study because like the impedance method it allows physiological examination of stapedius muscle function without modifying the muscle or the middle ear as is necessary in conventional strain gauge measurements

The sound stimulus was delivered ipsilaterally or contralaterally to the impedance measuring device by a procedure described by Møller (1961) The following acoustic stimuli were used (*a*) pure tones at 2 000 Hz up to 128 dB SPL (*b*) 1/1 or 1/3 octave noise centered at 2 000 Hz, (*c*) wide band noise EMG measurements were also made during natural stimulation such as human vocalization handclapping and chirping of other chickens in the sound field

The birds vocalized often and spontaneously a few hours after the initiation of the experiment. In other instances vocalization was induced by light mechanical pressure on the chest Animal vocalizations were measured at about 20 cm from the bird s beak by a ½-inch microphone (Brüel & Kjær 4133) and a Brüel & Kjær model 2607 sound level meter

RESULTS

A Acoustic reflexes

In none of the 7 birds tested were we able to induce a consistent EMG volume change or impedance change by pure tones narrow band or broad-band noise handclaps human vocal sounds or chirps by other chickens. There were two exceptions to this rule First ly in response to acoustic stimulation the preparation often started to vocalize especially when other on-experimental chickens in the immediate laboratory area were emitting characteristic distress chirps During this type of chirping response (which was apparently evoked reflexively" by chirps from other animals) the stapedius muscle was activated

Secondly above about 115 dB SPL noise and in some cases also pure tones elicited general jerks of the head and corresponding impedance changes However it is believed that these impedance changes were due to the movement of the impedance device in relation to the animal s head rather than to stapedius muscle contractions At stimulus levels up to about 110 dB SPL there were no impedance changes and those impedance changes ob-

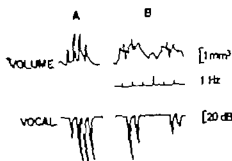


Fig. 3. Time course of volume change in the middle ear during chirping with both stapedius muscles intact (*A*) and after cutting muscles bilaterally (*B*).

graph seventy data points were averaged at 10 dB intervals from the lowest to the highest sound pressure levels on a single animal. This figure shows that the stapedius muscle is active at all levels of vocalization and it increases systematically as the chirp level increases.

Thus the volume of the middle ear cavity like the integrated EMG was found to change regularly during chirping (Fig. 2). In the intact animal with both stapedius muscles active the volume of the middle ear cavity increased 1–1.5 mm³ during chirping. Under the operating microscope the ear drum was observed to move in synchrony with stapedius movements and corresponding volume changes. It is important to note that the volume change was essentially equal in both ipsilateral and contralateral middle ears, even when one stapedius was blocked or destroyed. This demonstrates that there is a direct pressure communication between the two middle ear cavities.

In order to determine whether the observed volume changes were due exclusively to the contraction of the stapedius, the stapedial muscles were inactivated. After cutting both stapedius muscles the recorded volume change was markedly altered but clearly present during vocalization (Fig. 3A, B). Furthermore it was not possible to abolish this volume change even by additional removal of outer ear canal muscles and/or blocking of the eustachian tube.

These experiments indicate that the volume change in each middle ear is due to the ipsilateral stapedius muscle, the contralateral stapedius and to a lesser extent possibly the outer ear muscles or air pressed into the system via the eustachian tube or a deformation of the whole skull during vocalization. The importance of the influence of these factors for the control of sound transmission has yet to be investigated.

DISCUSSION

The present experiments show: (1) There is no specific acoustic reflex of the stapedius muscle of *Gallus gallus* (domesticus). (2) The stapedius is regularly activated during the animals own vocalization and its activity increases in a systematic fashion during increases in the chirp sound level. (3) There are mechanisms other than stapedius which influence middle ear function during chirping. (4) There is a pressure communication between the two middle ear cavities.

The avian order evidently differs from that of mammals in that it does not generally demonstrate a specific acoustic middle ear muscle reflex. It is to be noted that a specific acoustic reflex of the tensor tympani muscle only occurs in certain mammalian species and not for instance in humans and monkeys (Kato 1913). Also with respect to the stapedius muscle reflex there exist considerable species differences. Humans for example have a significantly higher acoustic reflex threshold than do lower mammals (Borg 1972). As indicated earlier Wada (1924) also failed to find acoustic activation of the stapedius of pigeons. This finding is in agreement with those of the present study. The variation among the avian species is further emphasized by the experiments of Golubeva (1972) on owls, in which both electromyographic and cochlear microphonic recordings demonstrated stapedius muscle activity during sound stimulation.

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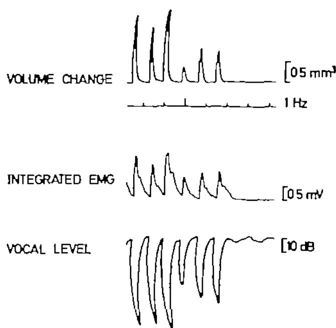


Fig A Time course amplitude relations of volume change in the middle ear (*top trace*) integrated EMG from the stapedius muscle (*middle trace*) and sound level of spontaneous chirps (*lower trace*) of a lightly anesthetized chicken Vocal deflection downward

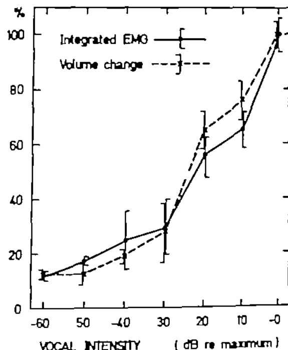


Fig 2 B Amplitude of integrated EMG from the stapedius muscle (—) and volume change ( ) in the middle ear in response to vocalization as a function of vocal sound level Average (±S E.M) in 10 dB intervals Each curve represents 70 data points

a bipolar electrode in the stapedius (upper records) and microphone recordings of the chirping sound during spontaneous vocaliza tion in a 4-week-old chicken In Fig 1 A a series of responses are illustrated on a slow time base to show the regularity in the cor relation of chirp sound and stapedius activ ity The inter-chirp intervals were generally quiescent and showed little or no spontaneous electrical activity In Fig 1 B a more detailed comparison of the EMG and the vocal sound is presented This figure shows that the EMG starts simultaneously with the chirp sound and builds up slowly to a constant level The elec trical activity in the stapedius was found to start an average 2 ms after the first oscillo-graphic sign of the vocalization sound (aver age from 54 recordings in 3 birds) In some of these recordings we obtained action potentials a few milliseconds before the start of vocaliza tion However this was not a consistent pat tern and such pre vocal activity was thought to be due to occasional spontaneous back ground activity

In some cases motor unit potentials were recorded along with the summated electrical activity by the extracellular electrode The width of the motor unit potentials was approximately 2 ms The motor unit potential frequency increased during the rise and peak of the vocalization At the beginning of the decay from the peak of the sound envelope there was often an inhibition of the EMG activity

In order to relate the magnitude of the stapedius activity to the animal s vocal sound level the amplitude of the integrated electromyogram and the amplitude of the volume change were examined

Fig 2 A shows a typical recording of the relationship of the middle ear volume change integrated EMG activity and vocalization level This figure demonstrates the relationship between the intensity of vocalization and the magnitude of stapedius activity

Fig 2 B shows the mean values for the amplitude of the integrated EMG and volume change in relation to chirp sound level In this

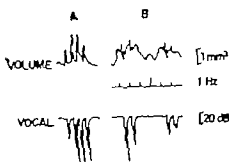


Fig. 3. Time course of volume change in the middle ear during chirping with both stapedius muscles intact (*A*) and after cutting muscles bilaterally (*B*).

graph seventy data points were averaged at 10 dB intervals from the lowest to the highest sound pressure levels on a single animal. This figure shows that the stapedius muscle is active at all levels of vocalization and it increases systematically as the chirp level increases.

Thus, the volume of the middle ear cavity like the integrated EMG was found to change regularly during chirping (Fig. 2). In the intact animal with both stapedius muscles active the volume of the middle ear cavity increased 1–1.5 mm³ during chirping. Under the operating microscope the ear drum was observed to move in synchrony with stapedius movements and corresponding volume changes. It is important to note that the volume change was essentially equal in both ipsilateral and contralateral middle ears, even when one stapedius was blocked or destroyed. This demonstrates that there is a direct pressure communication between the two middle ear cavities.

In order to determine whether the observed volume changes were due exclusively to the contraction of the stapedius, the stapedial muscles were inactivated. After cutting both stapedius muscles the recorded volume change was markedly altered but clearly present during vocalization (Fig. 3A, B). Furthermore it was not possible to abolish this volume change even by additional removal of outer ear canal muscles, and/or blocking of the eustachian tube.

These experiments indicate that the volume change in each middle ear is due to the ipsilateral stapedius muscle, the contralateral stapedius, and to a lesser extent possibly the outer ear muscles, or air pressed into the system via the eustachian tube, or a deformation of the whole skull during vocalization. The importance of the influence of these factors for the control of sound transmission has yet to be investigated.

DISCUSSION

The present experiments show: (1) There is no specific acoustic reflex of the stapedius muscle of *Gallus gallus* (domesticus). (2) The stapedius is regularly activated during the animals own vocalization and its activity increases in a systematic fashion during increases in the chirp sound level. (3) There are mechanisms other than stapedius which influence middle ear function during chirping. (4) There is a pressure communication between the two middle ear cavities.

The avian order evidently differs from that of mammals in that it does not generally demonstrate a specific acoustic middle ear muscle reflex. It is to be noted that a specific acoustic reflex of the tensor tympani muscle only occurs in certain mammalian species and not, for instance, in humans and monkeys (Kato, 1913). Also, with respect to the stapedius muscle reflex, there exist considerable species differences. Humans, for example, have a significantly higher acoustic reflex threshold than do lower mammals (Borg, 1977). As indicated earlier, Wada (1924) also failed to find acoustic activation of the stapedius of pigeons. This finding is in agreement with those of the present study. The variation among the avian species is further emphasized by the experiments of Golubeva (1977) on owls, in which both electromyographic and cochlear microphonic recordings demonstrated stapedius muscle activity during sound stimulation.

The activity of the stapedius muscle during vocalization is on the other hand well established in several mammalian species including humans (Borg & Zakrisson 1974) Unlike humans and bats (Henson 1965 Suga & Jen 1976) the stapedius of *Gallus* does not show electrical activity significantly before the start of the vocalization sound However since there is no acoustic reflex in chickens a non-acoustic route of activation must be operating Two possible routes are suggested (1) directly from the CNS center for vocalization or (2) reflexively from receptors in the syrinx or in the vocal tract Evidence exists in humans (Zakrisson & Borg 1977) that the larynx has to be intact if the stapedius is to be active during vocalization In certain mammals electrical stimulation of laryngeal nerves has been found to elicit activation of the stapedius (McCall & Rabuzzi 1973 Suga & Jen 1976)

In the bat the stapedius muscle contraction efficiently attenuates the emitted orienting sounds within the middle ear and relaxes in order to receive the echoes unattenuated (Henson 1965) Since such functional demands do not seem to exist in the chicken it is not surprising to find that stapedius activity does not start before the sound and that the muscles are not particularly fast in their contraction properties (Borg et al 1978)

The use of volume change as a measure of stapedius activity was found to be highly suitable in the present experiments It is similar to the impedance technique in allowing for examination of stapedius function under intact physiological conditions However unlike impedance measurements the measurement of volume change is unaffected by acoustic disturbances e g the animal s own vocal sounds

In conclusion stapedius muscle of *Gallus gallus* is regularly activated during vocalization The muscle s activity is initiated from the CNS vocal centres or by other non acoustic routs since there is no acoustic reflex mechanism for the stapedius in this species

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die Funktion des Mittelohrmuskels beim Vogel wurde an Hähnchen der Gattung Gallus gallus domesticus untersucht Die Aves bieten sehr günstige Vorbedingungen zum Studium der Mittelohrmuskelfunktion da sie nur einen einzigen Mittelohrmuskel nämlich den m stapedius besitzen der extrakranial belegen ist. Zur Bestimmung der Aktivität des Muskels wurden Elektromyogramme (EMGs) aufgenommen und Messungen der Impedanzänderung und der Volumenänderung der Mittelohrhöhle durchgeführt. Aus diesen Messungen ergab sich, daß der Mittelohrmuskel vom Gallus keinen akustischen Reflex zeigt Andererseits wird jedoch der m stapedius regelmäß g während der Eigenvokalisation des Tieres aktiviert. Aus den EMG- und Volumenänderungsmessungen war weiterhin zu ersehen daß die Stapediusaktivität systematisch mit der Zunahme des Schallpegels der Vokalisation ansteigt Die Volumenänderung erwies sich in den vorliegenden Untersuchungen als ein sehr geeignetes Maß der Stapediusfunktion da mit ihr das Umgebungsgeräusch und die rein akustische Auswirkung der Eigenvokalisation die bei der Impedanztechnik berücksichtigt werden müssen nicht beeinflußt werden.

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CONTRACTION PROPERTIES AND FUNCTIONAL MORPHOLOGY OF THE AVIAN STAPEDIUS MUSCLE

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Abstract The influence of the stapedius muscle contraction on middle ear volume and acoustic impedance was investigated in the chiken *Gallus gall* The time course of twitch responses to electrical stimulation (measured as volume and impedance changes) was found to be largely independent of the stimulus voltage having a contraction time of ⁓ ms and a half-relaxation time of ⁓ ms The stapedius muscle was therefore characterized as a fast twitch muscle Slow contraction properties were also revealed A summation of responses to repetitive stimulation beginning at 2.5 Hz and a slow decline to baseline were seen in volume and impedance change recordings The morphological characteristics were consonant with that of a homogeneously fast muscle Only fibres with high ATPase activity were identified and no fibres with "en grappe or multiple innervation were observed The slow characteristics were suggested to be due to visco-elastic elements in the middle ear The chicken stapedius muscle is suggested to be analogous to both the stapedius and the tensor tympani of mammals

Unlike that of mammals the middle ear of aves has a single muscle the stapedius associated with its ossicular structures This muscle is innervated by a branch of the facial nerve (Smith 1904 Pohlman 1921) The muscle originates in a small depression at the base of the occipital bone and runs anteriorly and ventro-laterally then turns medially to attach the extrastapedial portion of the columella and the edge of the tympanic membrane (Smith 1904 Pohlman 1921) The stapedius muscle of the chicken *Gallus gallus* contracts regularly during vocalization (Counter & Borg 1979) and in this respect it is similar to that of many mammalian species (Henson 1965 Borg & Zakrisson 1975) In contrast to mammals the stapedius of certain avian species has been found to show no acoustic reflex (Wada 1924 Counter & Borg 1979 cf Golubeva 1972)

At the present time several biological features of the middle ear muscle of birds are in need of fuller definition particularly the contraction properties and fibre composition The aim of this study was to determine the contraction properties and functional morphology of the stapedius muscle of *Gallus gallus* In order to examine the contraction properties under intact physiological conditions measurements of the change in middle ear volume and impedance were used rather than the conventional strain gauge approach The fibre composition was defined on the basis of ATPase staining and electron microscopy In addition the end plate morphology was analysed on the basis of cholinesterase stained specimens

METHODS

Male white leghorns from 4 to 12 weeks old were used For the impedance and volume measurements the animals were anesthetized with nembutal (60 mg/kg body weight)

Physiology

The head was mounted on a rigid head holder and the body held in a wire cage The stapedius was then surgically exposed and a stainless steel bipolar stimulating electrode was carefully placed on its surface by a micromanipulator Electrical rectangular pulses of 5 ms duration were generated by a Grass model S4 stimulator For measurements of middle ear volume change a 12-gauge metal cannula was inserted through the cranial bone into the

middle ear cavity near the tympanic ring and sealed. The cannula was connected to a plastic tube which led to a Grass model PT5A volumetric pressure transducer. This device measures volume changes without introducing significant pressure changes into the system. Dynamic pressure changes as small as 0.3 Pa (0.002 mmHg) can be observed by this transducer. Signals representing volume changes were recorded on a Grass 79D system (frequency = 60 Hz), a Tandberg FM tape recorder and a Siemens 34T ink recorder. For impedance measurements the ear canal was surgically removed and a 0.5 cm diameter rubber tube was mounted and sealed around the rim of the tympanic membrane. Acoustic impedance change was measured at 800 Hz in one or both ears simultaneously by a technique developed by Møller (1961). The level of the probe tone was 77 dB SPL (sound pressure level re. 20 μPa).

Morphology

For histochemical analysis stapedius muscles were frozen in isopentane previously cooled with liquid nitrogen to about −160°C. Sections 10–15 μm thick were cut in a cryostat at −20°C. The staining for myofibrillar adenosine triphosphatase (ATPase) was made according to Padykula & Herman (1955) at pH 9.4. In addition preincubation at pH 4.3 was performed (Dubowitz & Brooke, 1973).

Cholinesterase activity at the end-plate of the stapedius muscle was demonstrated by a modified thiolacetic acid method (Barrnett & Palade, 1959). 0.5 ml of concentrated thiolacetic acid (Sigma) was added to 10 to 15 ml of 0.1 M Na-cacodylate buffer and titrated to pH 5.5–6.0 with 1 M NaOH. The best results were obtained in the more acidic range. The solution was diluted to 20 ml with 0.1 Na-cacodylate buffer at the same pH as that of the titrated solution. Immediately before use 0.5 ml of 20 mM $Pb(NO_3)_2$ was added to 4.75 ml of the thiolacetic acid solution, which gave a final lead nitrate concentration of 1 mM. Visible deposits were obtained after 15–30 min.

To visualize the nerve innervating the stapedius muscle, 1% osmium tetroxide (OsO_4) in 0.1 M Na-cacodylate buffer was applied to the muscle.

For electron microscopy the muscles were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Na-cacodylate buffer at pH 7.1–7.3 to which was added 2 mM $CaCl_2$ and 2 mM $MgCl_2$. The muscles were postfixed in 2% OsO_4 and mordanted with 1% tannic acid (Simionescu & Simionescu, 1976). After dehydration in alcohol and embedding in Epon ultrathin sections were cut on LKB Ultratome, stained with uranylacetate and lead citrate and examined in a Zeiss EM9 electron microscope (see also Rydqvist, 1978).

RESULTS

Physiology

When the stapedius muscle was stimulated directly by single electrical pulses corresponding large volume and impedance changes were induced in the middle ear system. Fig. 1 shows superimposed single volume changes in response to a range of electrical stimuli from 2 to 12 volts. Monophasic increases in middle ear volume are seen, corresponding to a net outward movement of the tympanic membrane. The peak amplitude of the twitch response increases in an approximately linear fashion with increase in the stimulus voltage and finally reaches a plateau. The pattern of impedance change (Fig. 2 inset) is similar to that for volume change shown in Fig. 1. However the impedance change responses reached saturation at slightly lower stimulus voltage than the volume changes and usually showed a double peak at the highest stimuli. Another characteristic feature of the volume and impedance change recordings was the delay in the return to baseline.

A more detailed description of the time course of twitch responses is illustrated in Fig. 2. The contraction time (T_2–T_1, Fig. 2A) and the half relaxation time (T_4–T_3, Fig. 2B)

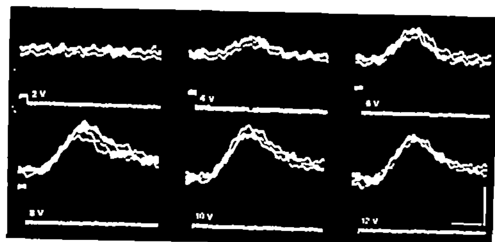
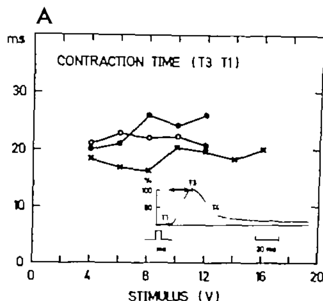


Fig 1 Superimposed single tracings representing volume increase in the middle ear cavity following electrical stimulation of ipsilateral stapedius muscle with rectangular 5 ms pulses. Vertical bar 1 mm³ change of volume Time bar 20 ms.

measured as volume changes are shown for 3 animals as a function of stimulus voltage The inset shows an averaged twitch response recorded as a change of acoustic impedance including the time intervals measured Fig 2 illustrates that the contraction time is largely independent of the stimulus strength The average contraction and half relaxation times measured as volume change were 22 (S D =3) ms and 22 (±1) ms respectively The corresponding values for impedance change were 22 (±3) and 22 (±14) ms respectively The latency (T_1) was on the average 13 ms for volume change and 11 ms for impedance change and was essentially independent of voltage T_2 (from start of stimulation to 50% of maximum response) was on the average 22 ms for both volume and impedance change Following T_4 a (typically) slow decline to baseline can be observed

When repetitive stimulation was used individual contraction responses were distinguished in the volume and impedance recordings up to about 40 Hz at which frequency complete fusion occurred In both volume and impedance responses to repetitive stimulation two components were seen (1) a rapid twitch-like component and (2) a slowly rising and decaying component Fig 3 shows both volume (3A) and impedance (3B) changes to



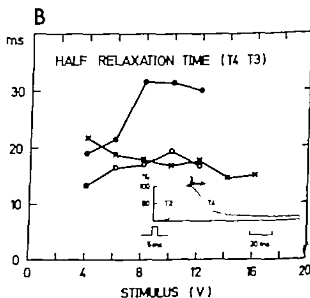


Fig 2 (A) Contraction time as a function of the voltage of 5 ms electrical pulse applied to the stapediu muscle in three chickens. Inset shows average impedance change response (n=1) with measured time lags indicated Arrow indicates contraction time (T_3–T (B) Half rel xation time (T_4–T_3) as indicated in Inset

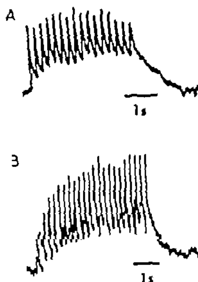


Fig. 3. Response to repetitive stimulation of stapedius muscle at 5 Hz. (A) Volume change. (B) Impedance change.

5 Hz stimuli in which the slow components are prominent. This slow feature was found to extend down *to 2.5 Hz stimulation. It* persisted for at least 1 sec after the end of stimulation. Frequently the slow component was somewhat more prominent in the recordings of the impedance change than in the volume change.

Morphology

In an attempt to further elucidate the slow and fast components of the physiological response a series of *morphological investigations* was undertaken. Morphological evidence for the presence of fast and slow fibres was examined first by the myosin–ATPase staining technique. As can be seen in Fig. 4A only heavily stained fibres were observed *(in pH 9.4).* In low pH (4.3) only lightly stained fibres were observed (not shown). These findings clearly indicate the presence of predominantly fast elements.

Further analysis of the muscle composition was made by electron microscopy. The electron micrograph shown in Fig. 4B illustrates three typical muscle fibres with similar characteristics. These fibres showed well developed sarcomeres (see *inset*), thin and straight Z lines, symmetrical A and I bands and a clear M line. Sarcoplasmic reticulum and transverse tubules were clearly evident in each sarcomere. *These findings* conform to the criteria used by several investigators to define fast fibres (Fernand & Hess 1969, Teig & Dahl 1971, Hirayama & Daly 1974).

The morphology of the innervation pattern *has been used to distinguish* between fast and



Fig. 4 (A). Cross section of stapedius muscle of chicken. ATPase stained at pH 9.4 (bar 100 μm). (B) Electron micrograph of longitudinal section of stapedius muscle (bar 5 μm). Inset shows details of sarcomere (bar 1 μm).

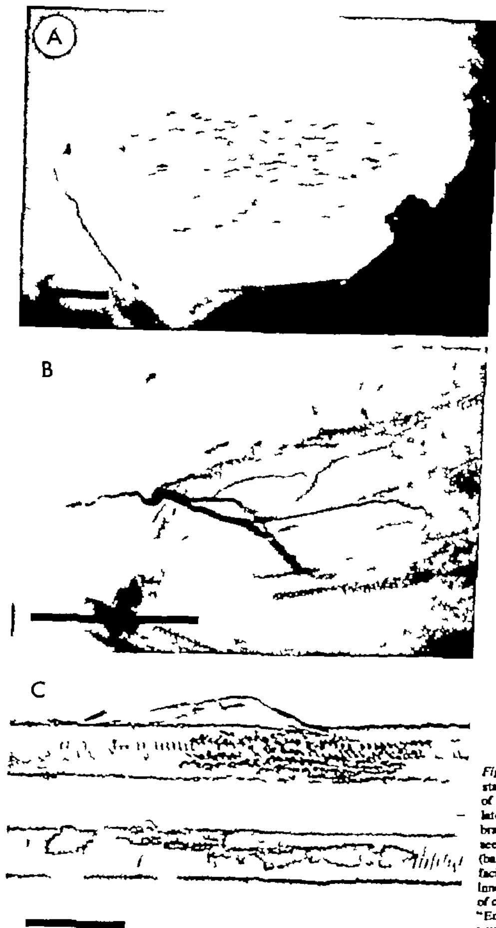


Fig 5 (A) Cholinesterase-stained whole stapedius muscle of chicken seen from a ventrolateral view Tympanic membrane to the left. End-plates are seen as elongated black spots (bar 1 mm) (B) Branch of the facial nerve (myelin-stained) innervating the stapedius muscle of chicken (bar 1 mm) (C) "En plaque" endings on two muscle fibres teased from the *stapedius muscle (bar 50 μm).*

low muscle fibres (Ginsborg & MacKay 1960). Fig. 5A shows the pattern of distribution of end-plates in the stapedius muscle in a cholinesterase-stained preparation. As is seen the end-plates are scattered over the entire muscle, which would tend to suggest the presence of multiply innervated muscle fibres (presumably slow). However, the abundant branching of the nerve fibre bundles (Fig. 5B) to different parts of the muscle belly may indicate that single end-plates are distributed over a wide area of the muscle. The individual innervation of single muscle fibres is substantiated by observations on cholinesterase stained, *teased muscle fibres. In none of 54* fibres teased from four muscles and inspected along their entire length did we observe more than one end-plate per fibre. The innervation of fast muscle fibres is generally accepted to be by "en plaque" endings, while that of slow fibres is usually by "en grappe" terminals. Fig. 5C show cholinesterase-stained end-plate regions of 2 muscle fibres. It can be seen that the elongated end-plates cover a substantial portion of the muscle fibre. The morphological features observed in these end-plates are consonant with the criteria for "en plaque" endings (Fernand & Hess 1969), thus implying fast contraction properties.

DISCUSSION

The contraction properties of the stapedius muscle of chicken clearly meet the criteria of *fast muscle*. The contraction time was found to be around 70 ms, as measured by middle ear volume and impedance changes. In addition, the physiological response patterns give evidence of slow components. This is most readily observed during the relaxation phase of the response and during repetitive stimulation at 2.5 Hz and above. The morphological analysis revealed a homogeneous muscle. Both the intensity of the ATPase staining and the presence of only "en plaque" neuromuscular junctions are compatible with the fast contraction properties observed (Bárány 1967, Teig & Dahl 1971). However, on the basis of the morphological findings it is difficult to account for the slow component of the responses described above.

There are three possible sources of this slow component:

1. the active contractile mechanism of the muscle fibres
2. non-contractile features and the topography of the whole muscle
3. the attachment of the stapedius muscle to the middle ear structure, i.e. the eardrum, the columella and the ligaments.

On the basis of the present findings, however, we were unable to clearly determine *which of these alternatives is the most plausible*. In studies presently underway, using conventional stain gauge measurements from the tendon close to the muscle belly, we observed no slow contraction properties (Borg et al. 1978). These findings implicate the third possibility, i.e. the properties of the attachments of the stapedius muscle to the middle ear (ligaments, structures, tendons and tympanic membrane) are primarily responsible for the slow activity.

It is reasonable to compare this muscle to the stapedius and tensor tympani of mammals. The mammalian middle ear muscles have an inhomogeneous fibre composition (Teig & Dahl 1971). There is physiological evidence of fast and slow twitch fibres (Teig, 1972) and at least morphological evidence for slow tonic fibres (Fernand & Hess 1969, Hirayama & Daly 1974) in cats and rabbits. This is in contrast to the chicken middle ear muscle which has a homogeneous fibre composition.

Interestingly, the physiological response patterns of the chicken stapedius revealed both fast and slow effects on the middle ear system in the *in situ* preparation. This information, coupled with the anatomical topography of the avian middle ear system would suggest that the single middle ear muscle of birds is functionally analogous to the two intra-aural muscles in mammals. The avian stapedius is attached to the stapes-like columella and is in this respect similar to the mam

malian stapedius in that they are both capable of controlling the vibration pattern at the oval window (Oecklinghaus & Schwartzkopff 1975) In addition like the mammalian tensor tympani it also directly influences the position of the eardrum and thus the pressure conditions in the middle ear

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ULTRASTRUCTURAL CHANGES OF THE NERVE ELEMENTS FOLLOWING DISRUPTION OF THE ORGAN OF CORTI

II *Nerve Elements Outside the Organ of Corti*

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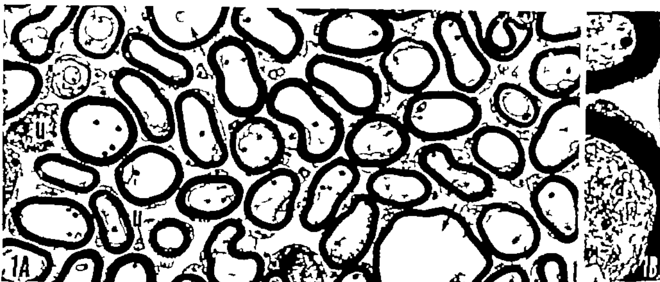


Fig 1 7 days after perfusion 20% SM Cochlear nerve fibers in the modiolus (A) Degenerating fibers (*arrows*) are scattered Among the myelinated fibers are a small number of unmyelinated fibers (*u*) ×5100 (B) Tubule-like organelles in the axoplasm (*a*) probably an early sign of degeneration ×10200

umes of dihydrostreptomycin and streptomycin sulfate diluted in Ringer's solution at two different concentrations 2% and 20%

In the first group 28 cochleae from 18 animals were perfused with 20% SM solution The postoperative survival time of the animals ranged from 3 to 137 days In the second group 8 cochleae from 4 animals were perfused with 2% SM solution by the same procedure used for the first group The survival

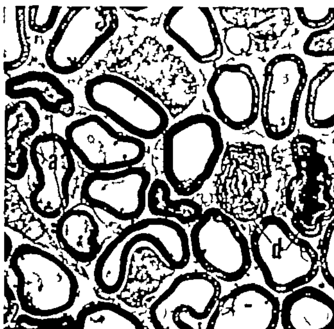


Fig 2 12 days after perfusion 20% SM Cochlear nerve trunk in the internal auditory meatus. The number of degenerating fibers has increased Some of them show evenly dense axoplasm (*a*) Others have lost axons and contain disintegrated myelin lamellae (*d*) An arrow indicates myelinating fiber with a thin myelin sheath ×5100

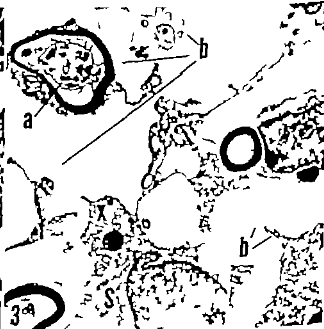


Fig 3 12 days after perfusion 20% SM A shrunken satellite cell (*S*) shows twig-like processes It is loosely surrounded by the basement membrane (*b*) In a branch (*a*) of a spiral ganglion cell dense bodies are accumulated *f* fibrocyte ×7200 The inset is an enlargement of a process (*x*) ×16000



Fig. 4. 17 days after perfusion, 20% SM. The cochlear nerve in the modiolus. Second turn. A Schwann cell (S) lost both axons and myelin lamellae. Arrows indicate Schwann cells still enclosing myelin debris. 6120.

time of the animals was 31 to 76 days. In the third group, 16 cochleae were perfused with Ringer's solution only. The animals were sacrificed 7 to 137 days postoperatively.

All specimens were fixed either by intra-aortic perfusion with 2.5% cacodylate buffered glutaraldehyde solution at pH 7.4 or by perilymphatic perfusion through the round window. The cochleae were removed and postfixed in 1% phosphate-buffered osmium tetroxide for one hour.

In this study, the specimens fixed by intra-aortic perfusion were mainly used because the nervous tissues outside the organ of Corti were insufficiently fixed by perilymphatic perfusion. The specimens were dehydrated in acetone and embedded in Epon 812. Sectioning was done with an LKB Ultrotome. The sections were double stained with uranyl acetate and lead citrate and were observed under : electron microscope.

FINDINGS

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Four days after perfusion, Schwann cells entered the organ of Corti through the habenula perforata.

Seven days after perfusion, nerve fibers in the collapsed organ of Corti were enclosed by Schwann cells. The axons of the fibers showed extension and sprouting. In the area central to the organ of Corti, macrophages were scattered among the spiral ganglion cells. Some of the ganglion cells exhibited shrinkage of their cell bodies and decrease of endoplasmic reticulum and Golgi apparatus. Infolding of the cell membrane was found. Satellite cells were also severely degenerated. In the osseous spiral lamina, modiolus and internal auditory meatus, clear and swollen axoplasm of the nerve fibers was randomly observed (Fig. 1A). Many elongated tubular organelles appeared in the axoplasm (Fig. 1B).

From 10 to 14 days after perfusion, the organ of Corti collapsed into a mass containing debris, tectorial membrane, macrophages, fibrocytes and remaining nerve fibers. These nerve fibers already developed into myelinated or unmyelinated fibers. In the modiolus and internal auditory meatus, macrophages began to infiltrate the nerve fibers. They digested various debris within the vacuoles in the cytoplasm. Degenerating nerve fibers were distributed evenly in the cochlear nerve. The degeneration was shown as clear and swollen axoplasm with lysis of mitochondria or as dense axoplasm (Fig. 2). Then the nerve fibers

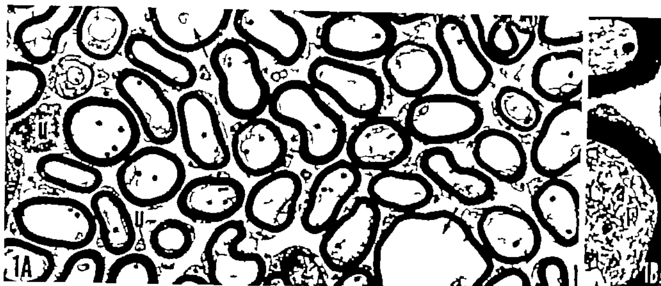
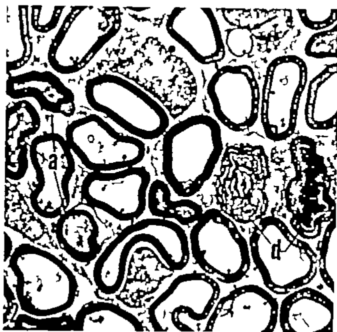


Fig 1 7 days after perfusion 20% SM Cochlear nerve fibers in the modiolus (A) Degenerating fibers (*arrows*) are scattered Among the myelinated fibers are a small number of unmyelinated fibers (*u*) ×5100 (B) Tubule-like organelles in the axoplasm (*a*) probably an early sign of degeneration ×10 700

umes of dihydrostreptomycin and streptomycin sulfate diluted in Ringer s solution at two different concentrations 2% and 20%

In the first group 28 cochleae from 18 animals were perfused with 20% SM solution The postoperative survival time of the animals ranged from 3 to 137 days In the second group 8 cochleae from 4 animals were perfused with 2% SM solution by the same procedure used for the first group The survival



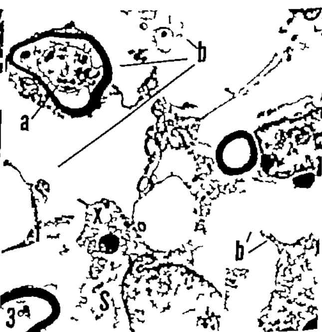


Fig 2 1 days after perfusion 20% SM Cochlear nerve trunk in the internal uditory meatus The umber of degenerating fibers has increased Some of them show evenly dense axoplasm ( ) Others have lost axons and contain disintegrated myelin lamellae (*d*) An arrow indicates myelinating fiber with a thin myeli sheath ×5100

Fig 3 12 days afte perfusion 20% SM A shrunken satellite cell (*S*) shows twig-like processes It is loosely surrounded by the basement membrane (*b*) In a branch ( ) of spiral ganglion cell dense bodies are accumulated *f* fibrocyte ×7200 The I set is an enlargement of a process (*x*) ×16000



Fig. 4. 17 days after perfusion 20% SM. The cochlear nerve in the modiolus. Second turn. A Schwann cell (S) lost both axons and myelin lamellae. Arrow indicates Schwann cells still enclosing myelin debris. ×6120

time of the animals was 31 to 76 days. In the third group, 16 cochleae were perfused with Ringer's solution only. The animals were sacrificed 7 to 137 days postoperatively.

All specimens were fixed either by intra-aortic perfusion with 2.5% cacodylate buffered glutaraldehyde solution at pH 7.4 or by perilymphatic perfusion through the round window. The cochleae were removed and postfixed in 1% phosphate-buffered osmium tetroxide for one hour.

In this study, the specimens fixed by intra-aortic perfusion were mainly used because the nervous tissues outside the organ of Corti were insufficiently fixed by perilymphatic perfusion. The specimens were dehydrated in acetone and embedded in Epon 812. Sectioning was done with an LKB Ultrotome. The sections were double stained with uranyl acetate and lead citrate and were observed under the electron microscope.

FINDINGS

The findings in animals perfused with 20% SM were as follows. Three days after perfusion, a few spiral ganglion cells showed myelin figures, vacuoles and granulated spherical bodies of their perikaryon as early signs of degeneration. The cochlear nerve fibers in the modiolus, however, did not yet show degeneration. The organ of Corti at this stage kept its normal contour but there was a loss of outer hair cells.

Four days after perfusion, Schwann cells entered the organ of Corti through the habenula perforata.

Seven days after perfusion, nerve fibers in the collapsed organ of Corti were enclosed by Schwann cells. The axons of the fibers showed extension and sprouting. In the area central to the organ of Corti, macrophages were scattered among the spiral ganglion cells. Some of the ganglion cells exhibited shrinkage of their cell bodies and decrease of endoplasmic reticulum and Golgi apparatus. Infolding of the cell membrane was found. *Satellite cells* were also severely degenerated. In the osseous spiral lamina, modiolus and *internal auditory* meatus, clear and swollen axoplasm of the *nerve fibers* was randomly observed (Fig. 1A). Many elongated tubular organelles appeared in the axoplasm (Fig. 1B).

From 10 to 14 days after perfusion, the organ of Corti collapsed into a mass containing debris, tectorial membrane, macrophages, fibrocytes and remaining nerve fibers. These nerve fibers already developed into myelinated or unmyelinated fibers. In the modiolus and internal auditory meatus, macrophages began to infiltrate the nerve fibers. They digested various debris within the vacuoles in the cytoplasm. Degenerating nerve fibers were distributed evenly in the cochlear nerve. The degeneration was shown as clear and swollen axoplasm with lysis of mitochondria or as dense axoplasm (Fig. 2). Then the nerve fibers

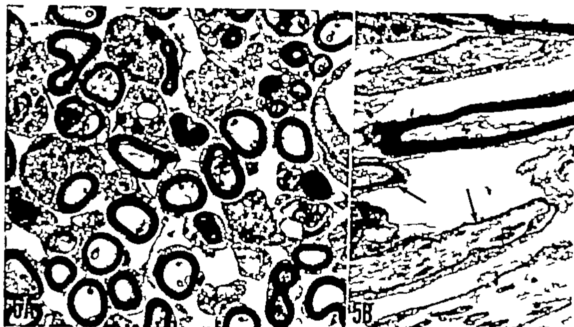


Fig 5 Nerve fibers in the osseous spiral lamina of the second turn Spiral section (A) 17 days after perfusion 70% SM Fiber population is still dense Nerve fibers in various stages of degeneration and regeneration are observed A few fibers show axonal sprouting associated with myelin debris (*arrows*) Regenerating myelinated fibers cannot be distinguished from proper unmyelinated fibers unless their Schwann cells still contain myelin debris ×3470 (B) 20 days after perfusion 70% SM Two fibers (*arrows*) spirally coursing have thin myelinating sheaths. ×4500

which showed disappearance of axons and disrupted myelin lamellae increased in number (Fig 2) The Schwann cells displayed hypertrophy The population of nerve fibers in the osseous spiral lamina and cochlear nerve trunk was still dense

Although very rare some fibers began myelination of the newly developed axons 12 days after SM perfusion They were recognizable by the thin myelin sheath encircling the axon (Fig 2) as described below The degenerative changes in the spiral ganglion cells and their satellite cells were more prominent than in the earlier stage They often shrank into a mass composed only of protoplasm with twig like processes and were loosely surrounded by basement membrane (Fig 3) In this stage spiral ganglion cells with normal appearance were still frequently encountered

In a 14-day specimen a small number of degenerating nerve fibers was also observed in the olivo-cochlear bundle Among the intact nerve fibers several shrunken Schwann cells with loose and folded basement membranes were found

From 17 to 20 days after SM perfusion the nerve fibers remaining in the organ of Corti did not show any signs of degeneration and maturation was in progress In the cochlear nerve trunk Schwann cells which had lost their axons and myelin lamellae increased (Fig 4) Throughout the areas observed empty spots were found sparsely distributed among the degenerating nerve fibers and ganglion cells Fiber density in the osseous spiral lamina was still unchanged but the number of intact fibers was reduced (Fig 5 A)

In a 20-day specimen signs of regeneration were prominent Numerous Schwann cells enclosed multiple sprouts of newly developed axonal processes and occupied the spaces between myelinated fibers Thus the cochlear nerve which is normally composed of almost exclusively myelinated fibers appeared to be mainly composed of unmyelinated fibers (Fig 6) Some of the regenerating fibers showed a

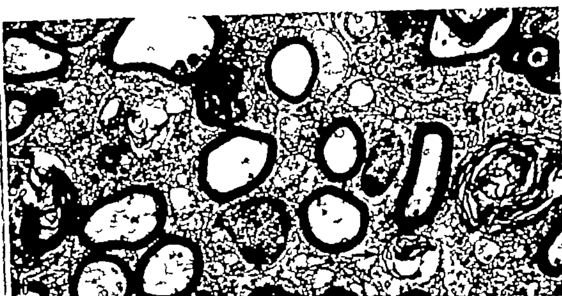


Fig. 6. 20 days after perfusion, 20% SM. Cross section of the cochlear nerve trunk in the internal auditory meatus shows stage of axonal sprouting. The cochlear nerve which normally consists of myelinated fibers appears to be mixed with unmyelinated fibers. Co-existence of myelin debris and axonal sprout in the same Schwann cell (arrow) is occasionally seen. ×6000

co-existence of axonal sprouts with debris of myelin lamellae derived from the original myelin sheath within the same Schwann cell (Figs. 6, 8). The co-existence of myelin debris made it easy to distinguish regenerating myelinated fibers from unmyelinated fibers. In the osseous spiral lamina, nerve fibers with axonal sprouts and myelinating fibers were often found in both radial and spiral fibers (Figs. 5A, 5B). The myelin lamellae of the myelinating fibers were still small in number.

Until this period the nerve fibers of the olivo-cochlear bundle in Rosenthal's canal and internal auditory meatus demonstrated only a small number of degenerated fibers. Fiber density of the bundle did not appear to be greatly reduced (Fig. 7A). Only a few myelinated fibers showed signs of regeneration



Fig. 7. Efferent fibers in the intraganglionic spiral bundle in the first turn, 20% SM. Only a few cochlear nerve fibers (C) survive. (A) 20 days after perfusion. Fiber population is still dense. ×1890. (B) 35 days after perfusion, 20% SM. Decrease in density of fibers is prominent. G: shrinking ganglion cell. ×1890.

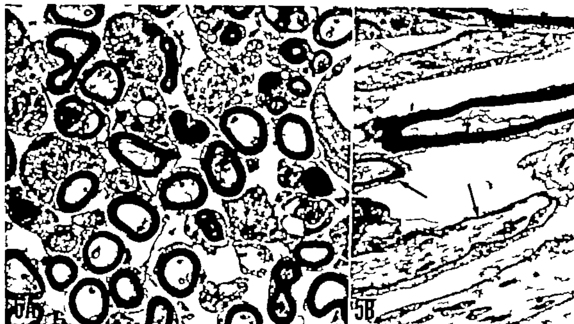


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Fig. 10. 137 days after perfusion 20% SM. Cross section of the cochlear nerve in the internal auditory meatus. Note diminution of fibers and shrinkage and vacancy of endoneurial compartments (*C*). ×17 930.

cochlear nerve trunk was reduced in diameter. The efferent olivo-cochlear bundle could not be found in the internal auditory meatus of the 137-day specimen. In Rosenthal's canal a small number of ganglion cells and nerve fibers still remained. They were scattered in an almost empty space with fibrocytes and fine collagen fibrils (Fig. 11).

The organ of Corti of the control animals perfused with Ringer's solution or 2% SM did not show collapse. The outer and inner hair cells were missing and were replaced by supporting cells. Although nerve fibers were markedly reduced in number, regeneration of the nerve fibers and invasion of Schwann cells into the organ of Corti were not found.

In the areas central to the organ of Corti of the control animals, degeneration and disappearance of the cochlear nerve fibers and ganglion cells were only sporadic. Degenerative and regenerative processes of the nerve fibers and ganglion cells were the same as those after perfusion with 20% SM, but in lesser extent and milder grade. Regenerating fibers were more frequently found in the modiolus and internal auditory meatus than in the 20% SM group. In the olivo-cochlear bundle degenerating fibers were rarely found (Fig. 12).

DISCUSSION

Degenerative and regenerative processes of myelinated and unmyelinated fibers of the peripheral nerves at the fine structural level have been already established in various kinds of nerves of various species (Wechsler & Hager 1962, Nathaniel & Pease 1963*a*, 1963*b*, Wettstein et al. 1963, Lampert, 1967, Haftek & Thomas 1968, Dyck & Hopkins 1972, Bray & Aguayo 1974, Murray 1976). The results of

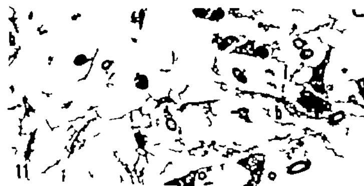


Fig. 11. 137 days post perfusion with 20% SM. Radial section of Rosenthal' canal, second turn. The area (*I*) was previously occupied by the intraganglionic spiral bundle. A small number of nerve fibers still survives. Thicker fibers are afferent, thinner are efferent. In this area, no ganglion cells remain. *b*: bone. ×11 315

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Fig 8 35 days after perfusion 70% SM A cross section of the cochlear nerve trunk in the intern auditory meatus shows stage of p myelination. Note decrease in nu ber of axon sprouts enclosed in a single Schwann cell as a preparati for the myelination of one of the sprouts in a single Schwann cell. Thin arrows indicate myelin debr and thick arrows disappearing fib with loose basement membranes. *m* myelinating fiber ×7470

It was not possible to identify the regenerating unmyelinated fibers in the olivo-cochlear bundle because both regenerating myelinated and unmyelinated fibers at the stage of axonal sprouting were similar in appearance to normal unmyelinated fibers as reported by other authors (Dyck & Hopkins 1972 Bray & Aguayo 1974)

In a 35-day specimen the nerve fibers remaining in the organ of Corti had completely matured into myelinated or unmyelinated fibers The latter often contained clusters of cored and agranulated vesicles in the axoplasms

In the cochlear nerve trunk regenerating unmyelinated fibers were still abundant but most of them enclosed only one or two axons as shown in Fig 8 Such decrease in number of axons in a Schwann cell would indicate a suppression of surplus axonal sprouts in the premyelination stage Myelinating axons we more frequently found than in the earlier sta (Figs 8 9) Despite the fact that regeneratio was in progress the total number of cochle nerve fibers and ganglion cells steadily d creased throughout the observed areas Th nerve fibers in the olivo-cochlear bundle e pecially unmyelinated fibers also becam markedly sparse during this period (Figs 7E 11)

In the 64- and 137-day specimen the ma tured fibers remaining in the organ of Corti be came thinner probably due to atrophy In th modiolus and internal auditory meatus th number of cochlear nerve fibers was so re duced that the endoneurial compartments i which bundles of nerve fibers were bordere by processes of fibrocytes became very smal and frequently contained only a few nerv fibers or none at all (Fig 10) Thus the whol



Fig 9 64 days after perfusion 70% SM Cross section of the cochlear nerve i the internal auditory meatus showing shrinkage of endoneurial compartments and new myelinated fibers with thin myelin sheath (*arrows*) *M* macrophage *f* fibrocytes as endoneurial border ×5575

nerves composed of purely myelinated or unmyelinated fibers since both myelinated and unmyelinated fibers mimic normal unmyelinated fibers at a certain stage of regeneration Thus regenerating fibers are difficult to identify in a mixed nerve

In our present study changes of the cochlear nerve fibers following disruption of the organ of Corti correspond well to the degenerative and regenerative changes observed in other peripheral nerve fibers Contrary to nerves injured by crushing however the regenerated nerve fibers in this experiment survived only temporarily The nerve fibers in the organ of Corti osseous spiral lamina, modiolus and internal auditory meatus progressively atrophied and decreased in number before and after maturation

The modality of regeneration in the organ of Corti and in the areas central to this structure seemed somewhat different. In the disrupted organ of Corti surviving nerve axons extended were ensheathed by Schwann cells and matured whereas, in the modiolus and internal auditory meatus the axons of the cochlear nerve fibers disappeared and then new axonal sprouts extended into the band of Buengner This is possibly one reason why the regenerative process in the organ of Corti precedes that outside the organ

Identification of nerve fibers regenerated in the disrupted organ of Corti is of interest since if regenerating fibers are afferent they might be available for electrical stimulation by means of cochlear implant Johnsson and Hawkins (1977) and Wright (1976) using light microscopy found a few myelinated fibers in the degenerated organ of Corti of the gentamycin-intoxicated guinea pig, Dalmatian dog, and noise-exposed guinea pig They thought that the myelinated nerve fibers would regenerate from spiral ganglion cells Lim (1976) using the electron microscope observed regenerated unmyelinated nerve fibers in the osseous spiral lamina after kanamycin intoxication and assumed that the fibers were derived from type II unmyelinated spiral ganglion cells After transection of the cochlear nerve in the internal auditory meatus of cats Spoendlin & Suter (1976) found much proliferation of the nerve fibers in the organ of Corti and the regenerating myelinated fibers crossing the spiral ganglion cells They considered that the former fibers showed a ramification of afferent terminals and the latter were efferent fibers

In our previous study (Terayama et al 1977) we thought that the regenerating myelinated and unmyelinated fibers in the collapsed organ of Corti were mainly the terminal portions of the efferent olivo-cochlear bundle since the nerve cells which are the nutritional source of the bundle would remain intact in the brain stem and the regenerated unmyelinated axons showed typical features of efferent fibers such as varicose enlargements and accumulation of vesicles

In the present study regenerating myelinated fibers were randomly scattered in the intraganglionic spiral bundle and in the spirally coursing fibers in the osseous spiral lamina. In the osseous spiral lamina afferent and efferent fibers take radial and spiral courses respectively (Terayama et al 1969) These findings therefore support our previous assumption on regeneration of the efferent fibers

At the later stage however prominent decrease of nerve fibers in the olivo-cochlear bundle was observed This means that retrograde degeneration occurred in these fibers as previously suggested (Spoendlin 1975 Terayama et al 1977) In the control cochleae perfused with 2% SM most of the nerve fibers in the olivo-cochlear bundle survived although nerve fibers in the organ of Corti were markedly reduced in number Therefore the extent of the retrograde degeneration seems to depend upon the grade of damage to the organ of Corti

As to the cochlear nerve fibers regeneration could be easily recognized in the modiolus and internal auditory meatus since the cochlear nerve in these areas follows a separate course from other nerves and is almost exclusively



Fig 12 50 days postperfusion with 5% SM as a control Cross section of the cochlear nerve (*C*) and efferent olivo-cochlear bundle (*E*) in the internal auditory meatus Fiber population of the olivo-cochlear bundle is almost unchanged. ×3485

these studies are summarized as follows and illustrated in Fig 13 by our findings As the first sign of degeneration of myelinated fibers the axon of a nerve fiber becomes swollen and empty or evenly dense (Fig 13-2) Then the axon disappears and the myelin sheath disintegrates (Fig 13-3) If degeneration proceeds the Schwann cell decrease in size (Fig 13-4) and finally disappears leaving only the basement membrane (Fig 13-5) which also disappears later If the nerve fiber survives and regenerates multiple axonal sprouts extend into the tube of the basement membrane and remaining Schwann cells The tube is called band of Buengner (Figs 13-6 7) Therefore the regenerating myelinated fibers in this stage appear as if they are unmyelinated fibers which normally enclose many axons in a single Schwann cell Only one of these axons however becomes myelinated (Fig 13-8) and the remainder are suppressed and absorbed The regenerative process of unmyelinated nerve fibers is principally similar in myelinated ones except for myelination and number of axons in a Schwann cell (Dyck & Hopkins 1972; Bray & Aguayo 1974) Further maturation completes the regeneration by increasing the diameter of axons and number of myelin lamellae

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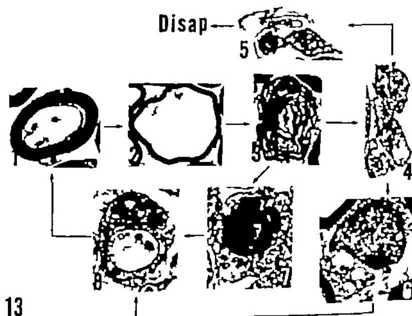


Fig 13 Sequential representation of degeneration and regeneration processes of the cochlear nerve fibers For details see text 1 3-4-5 degeneration 4-6-8-1 and 3-7 8-1 regeneration and maturation. *Disap* disappearance

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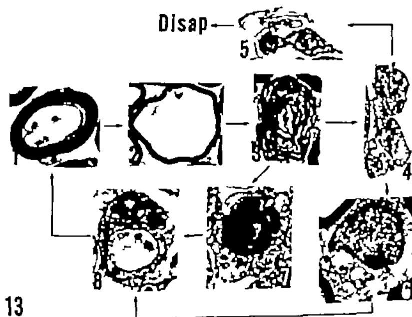


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LONG-TERM ELECTRODE IMPLANTATION FOR RECORDING COCHLEAR ELECTRICAL ACTIVITY IN GUINEA PIGS

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Abstract Comparative measurements of the cochlear electrical activity over prolonged periods require long-term implantation of electrodes. During this time, changes in the electrode recording ability and/or impairment of the sound conducting system, could erroneously be attributed to the experiment. To avoid this risk, an easy and reliable technique is described. The procedure uses the Fallopian canal as a route by which to approach the cochlear potentials' generators, while leaving the middle ear untouched

The recording of cochlear electrical activity in animals by various methods already described should and in fact does satisfy any researcher Exceptions to the rule however are studies with implanted electrodes in which measurements have to be made and compared over prolonged periods.

Spoendlin & Baumgartner (1977) mentioned that permanently implanted electrodes posed considerable problems However in prolonged experiments one must often use implanted electrodes despite the complex problems which arise The most critical requirement is absolute reliability in test–retest recordings during several months after the implantation. The most common site used for placement of the electrode is the round window but the opening of the bulla, the presence of a foreign body in the ear's air cells system and the prolonged contact between the electrode and the round window membrane represent a source of complication

Alteration in the electrode itself in the recording site or in the stability of their mutual contact, will induce changes in the recorded potentials which could erroneously be attributed to the on-going experiment. To avoid this risk, Spoendlin & Baumgartner (1977) placed the electrode in a small pit drilled close to the medial rim of the round window Because of the unavoidable thickening of the mucosa around the electrode tip they preferred to reopen the bulla and to place the electrode in the same location before each recording.

Of the disturbances which could occur over a long period of experimentation the following should be borne in mind

1 The path of the electrode which emerges through the skin represents a route for infection and if the bulla is opened, propagation to the middle ear is always possible

2. The animals tissue reaction to the foreign body could impair the middle ear function (thickening of the mucoperiostium of middle ear walls and/or ossicles bony growth effusion)

3 Less frequent but still possible displacement of the electrode tip from its original position

4 Damage of the electrode insulation due to movement of the surrounding muscles

5 Broken electrode

Our idea was to use the Fallopian canal as a way of introducing the electrode closer to the eighth nerve Being a closed bony canal the tissue reaction to foreign bodies is limited to

composed of myelinated fibers On the other hand there are several difficulties in identifying the kind of regenerating nerve fibers in the area peripheral to the spiral ganglion because afferent and efferent as well as myelinated and unmyelinated fibers are mixed The nerve fibers radially coursing in the osseous spiral lamina exhibited regeneration This finding strongly suggests that the cochlear nerve fibers regenerated not only in the central direction but also in the peripheral from the spiral ganglion cells However this study could not clarify the relationship between regenerated fibers and ganglion cells Furthermore it could not be ascertained whether the fibers actually reached the organ of Corti or not

The present study revealed that some fibers in the cochlear nerve and efferent olivo-cochlear bundle could regenerate following degeneration The regenerated cochlear nerve fibers however survived only temporarily and finally disappeared Thus these fibers would not be able to serve a useful purpose for electric stimulation

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ZUSAMMENFASSUNG

Verschiedene Stadien der Veränderungen der Nervenfasern Zellen des Spiralganglions und Satelhtzellen der Meerschweinchenschnecke wurden 3 bis 137 Tage nach perilymphatischer Perfusion mit Streptomyzinlösungen (5% und 20%) elektronenmikroskopisch beobachtet. Anfänglich waren die Axoplasm de Cochlearisnervenfasern geschwollen oder pyknotisch Dann verschwanden die Axone und zersplitterten die Myelinhülle. Die Schwannschen Zellen schrumpften und degenerierten während ihre Basalmembranen länger überlebten Regeneration der Cochlearisnervenfasern begann mit Streckung der Axonsprossen in die Röhre der Basalmembran und überlebender Schwannschen Zellen die noch Myelindebri enthalten Nur eines der Axonsprossen reifte zur Myelinisierung. Diese regenerierten Cochlearisfasern wurden n Lamina spiralis ossea Modiolus und innerem Gehörgang gefunden aber die Regenerate at roph erten und verschwanden später Retrograde Degeneration geschah in die olivo-cochleäre Bündel. Einige der efferenten Markfasern auch zeigten vorübergehende Regeneration.

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LONG-TERM ELECTRODE IMPLANTATION FOR RECORDING COCHLEAR ELECTRICAL ACTIVITY IN GUINEA PIGS

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Abstract Comparative measurements of the cochlear electrical activity over prolonged periods require long-term implantation of electrodes. During this time changes in the electrode recording ability and/or impairment of the sound-conducting system, could erroneously be attributed to the experiment. T avoid this risk, an easy and reliable technique is described The procedure uses the Fallopian canal as route by which to approach the cochlear potentials generators while leaving the middle ear untouched

The recording of cochlear electrical activity in animals by various methods already described should and in fact does satisfy any researcher Exceptions to the rule however are studies with implanted electrodes in which measurements have to be made and compared over prolonged periods

Spoendlin & Baumgartner (1977) mentioned that permanently implanted electrodes posed considerable problems However in prolonged experiments one must often use implanted electrodes despite the complex problems which arise The most critical requirement is absolute reliability in test–retest recordings during several months after the implantation. The most common site used for placement of the electrode is the round window but the opening of the bulla, the presence of a foreign body in the ear's air cells system and the prolonged contact between the electrode and the round window membrane represent a source of complication.

Alteration in the electrode itself in the recording site or in the stability of their mutual contact will induce changes in the recorded potentials which could erroneously be attributed to the on-going experiment. To avoid this risk Spoendlin & Baumgartner (1977) placed the electrode in a small pit drilled close to the medial rim of the round window Because of the unavoidable thickening of the mucosa around the electrode tip they preferred to reopen the bulla and to place the electrode in the same location before each recording

Of the disturbances which could occur over a long period of experimentation, the following should be borne in mind

1 The path of the electrode which emerges through the skin represents a route for infection and if the bulla is opened, propagation to the middle ear is always possible

2 The animals tissue reaction to the foreign body could impair the middle ear function (thickening of the mucoperiostium of middle ear walls and/or ossicles bony growth effusion)

3 Less frequent but still possible displacement of the electrode tip from its original position

4 Damage of the electrode insulation due to movement of the surrounding muscles

5 Broken electrode

Our idea was to use the Fallopian canal as a way of introducing the electrode closer to the eighth nerve. Being a closed bony canal the tissue reaction to foreign bodies is limited to

composed of myelinated fibers On the other hand there are several difficulties in identifying the kind of regenerating nerve fibers in the area peripheral to the spiral ganglion because afferent and efferent as well as myelinated and unmyelinated fibers are mixed The nerve fibers radially coursing in the osseous spiral lamina exhibited regeneration This finding strongly suggests that the cochlear nerve fibers regenerated not only in the central direction but also in the peripheral from the spiral ganglion cells However this study could not clarify the relationship between regenerated fibers and ganglion cells Furthermore it could not be ascertained whether the fibers actually reached the organ of Corti or not

The present study revealed that some fibers in the cochlear nerve and efferent olivo-cochlear bundle could regenerate following degeneration The regenerated cochlear nerve fibers however survived only temporarily and finally disappeared Thus these fibers would not be able to serve a useful purpose for electric stimulation

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ZUSAMMENFASSUNG

Verschiedene Stadien der Veränderungen der Nervenfasern Zellen des Spiralganglions und Satellitzellen der Meerschweinchenschnecke wurden 3 bis 137 Tage nach perilymphatischer Perfusion mit Streptomyzinlösungen (2% und 20%) elektronenmikroskopisch beobachtet Anfänglich waren die Axoplasm der Cochlearisnervenfasern geschwollen oder pyknotisch Dann verschwanden die Axone und zersplitterten die Myelinhülle Die Schwannschen Zellen schrumpften und degenerierten während ihre Basalmembranen länger überlebten Regeneration der Cochlearisnervenfasern begann mit Streckung der Axonsprossen in die Rohre der Basalmembran und überlebender Schwannschen Zellen die noch Myelindebris enthalten Nur eines der Axonsprossen reifte zur Myelinisierung Diese regenerierten Cochlearisfasern wurden in Lamina spiralis ossea Modiolus und innerem Gehörgang gefunden aber die Regenerate atrophierten und verschwanden später Retrograde Degeneration geschah in die olivo-cochleare Bündel. Einige der efferenten Markfasern auch zeigten vorübergehende Regeneration

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Fig. 2. A typical recording of A.P. obtained from the electrode placed in the facial canal (unfiltered click at 80 dB S.P.L. sound field). Upper trace: direct recording. Middle trace: average of 32 presentations. Lower trace: time scale.

nerve was identified and cut at its emergence from the bony canal. A 4 cm length of stainless steel thread for bony suture (3/0 usp multifilament surgical steel, Ethicon, Edinburgh) was prepared by covering it with a polyethylene tube, leaving the two ends free. A drop of histoacryl (B. Braun Melsungen A.G.) attached the polyethylene tube to the wire. A thick needle No. 18×1½ passed from the vertex under the muscles was brought out in the superior part of the incision near the opening of the facial canal (Fig. 1).

One end of the electrode was inserted into the needle, and by pulling it out, the electrode was brought outside the skin in the desired place. The proximal uninsulated end was cut to 4.5 mm, bent at approximately 60° and slowly introduced into the facial canal. There was no need to empty the canal by cauterizing the nerve.

We swabbed the opening of the facial canal and a drop of histoacryl fixed the electrode in place with the polyethylene tube to the bony surface. Two separate wire loops passing through the subcutaneous tissue in the neck region were used as reference and ground electrodes. For recordings performed a week after implantation, there was no necessity to sedate the animal.

COMMENTS

The spread of acoustically evoked potentials through the non-homogeneous volume conductor of the temporal bone is very complex. The pattern and magnitude of the cochlear action potentials complex change with the recording site, as earlier reported (Durrand & Ronis 1975, Aran & De Sauvage 1977, Daigneault 1974). The action potentials recorded with the technique described by us is shown in Fig. 2.

Compared with the previously described method, the improved technique developed by our group presents decided advantages. The middle ear, air cell system and external auditory meatus remain untouched. It is easy and rapid to perform, and relatively thick and resistant electrodes can be used.

The common limitation of these procedures (the previous and the presently described one) is that it can be used only in studies requiring implantation of electrodes in one ear only.

Sacrificing the facial nerve on both sides seriously impairs mastication, inducing, in most of the animals, a progressive deterioration of their general condition.

ZUSAMMENFASSUNG

Vergleichende Messungen der elektrischen Aktivität der Cochlea, die längere Zeit erfordern, verlangen eine langwährende Einpflanzung der Elektroden. Änderungen in der Elektroden-Aufnahmefähigkeit und/oder Minderung des Tonübertragungssystems während dieser Zeit konnten sonst fälschlicherweise mitgerechnet werden. Um diesen Fehler zu vermeiden, wird eine einfache und bewährte Methode beschrieben. Der Fallopian-Kanal wird benutzt, um der "Cochlear Potential"-Quelle nah zu kommen und das Mittelohr unberührt zu lassen.

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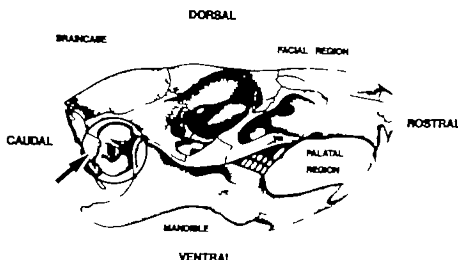


Fig 1a The circle encloses guinea pig s temporal bone. The arrow indicates the stylomastoid foramen through which the electrode is introduced

its content and the same holds true for some mild occasional infection propagating from outside along the electrode pathway Besides this isolated canal passes at certain points very near to the potentials generators thus facilitating a sensitive and faithful recording

A week after the implantation fibrous adhesions fixed the electrode to its walls ensuring stable contact and recording even in the presence of very strong acoustic vibrations Mismanagement of the electrode by the animal itself or occurring accidentally during experiments will not cause displacement

Rubinstein et al (1975) described a technique which in our opinion could avoid most of the difficulties but still required opening of the superior bulla During recent years we improved the technique making it easier to perform but conserving its main advantages, namely proximity to the potential generators and avoidance of the middle ear structures. We therefore consider it worthwhile to describe its simplicity and good and constant results

PROCEDURE

The guinea pig chosen for this experiment was sedated with Nembutal and under local anesthesia with Novocain a 2 cm incision was made in the retroauricular sulcus The facial

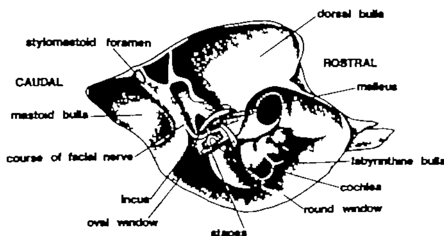


Fig 1b Opened bulla exposes th course of the facial nerve canal, forming the upper border of the oval window The tip of the electrode should reach this porti of the Fallopian canal (Redrawn from. *Anatomy of the G nea Pig* Cooper G & Schiller A L. Harvard Uni ersity Press Cambridge Mass. 1975)

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EFFECTS OF LOCAL APPLICATION OF OTOTOXIC ANTIBIOTICS ON COCHLEAR POTENTIALS IN GUINEA PIGS

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Abstract The effects of neomycin, kanamycin and dihydrostreptomycin on the cochlear microphonic, the action potential of the auditory nerve and the endocochlear potential were studied in guinea pigs in which these drugs were locally administered by perfusion. These drugs suppressed the cochlear responses markedly when applied to the endolymph but were less effective when applied to the perilymph. The mechanism of action of antibiotics on the hair cells of the organ of Corti are discussed.

These antibiotics suppressed the cochlear microphonics (CM) when applied to the scala media in a very low concentration whereas the CM was only gradually suppressed by the perilymphatic administration of relatively high concentration.

The ototoxicity of aminoglycosides has been well documented The consensus of the numerous papers points to the sensory cells of the organ of Corti as the site of primary damage However the route by which aminoglycosides reach the hair cells of the organ of Corti has not yet been well established and conflicting results have been reported to date

Stupp et al (1966 1973) concluded that the organ specificity of the ototoxic antibiotics is due to their accumulation in the inner ear fluids as a result of slow efflux out of the perilymphatic space On the other hand, Matsuura et al (1971 *a*) examined the effects of streptomycin and kanamycin on the microphonic potential of goldfish sacculus and found that these antibiotics suppressed microphonics only when administered intraluminally to the sacculus Similar observations have been reported in the canal organ (Wersäll & Flock, 1964) and in the excised semicircular canal of the frog (Harada et al 1967)

This study was designed to examine different effects of neomycin kanamycin and dihydrostreptomycin on the cochlear potentials in guinea pig cochlea following their perilymphatic or endolymphatic administration

METHODS

Healthy guinea pigs (NIH strain) weighing 300 to 400 g were anesthetized with pentobarbital sodium and the sound-evoked cochlear responses were recorded with differential electrodes (Tasaki et al 1952) The technique for recording the CM the action potentials of the auditory nerve (AP) and the endocochlear potential (EP) have been fully described previously (Butler et al. 1960) In most cases the AP in response to each of ten successive acoustic stimuli was averaged by a computer In experiments which involved perilymphatic perfusion, the cochlear potentials were recorded from the basal turn of the cochlea. When endolymphatic perfusion was performed the cochlear potentials were recorded from the second turn. The tone bursts used as acoustic stimuli were delivered in a closed system. The system was calibrated by measuring the sound pressure in front of the tympanic membrane with a probe tube and a calibrated condenser microphone.

Perilymphatic perfusion

The perfusion technique employed was similar to that described elsewhere (Konishi &

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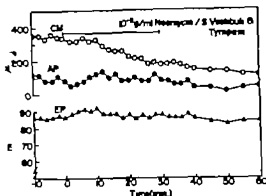


Fig 1 Effects of neomycin (10⁻ g/ml) on the cochlear microphonic (*CM*) and the action potential (*AP*) (top panel) and the endocochlear potential (*EP*) (bottom panel) Neomycin was applied to the scala vestibuli and tympani for period denoted by the horizontal line All responses were recorded from the basal turn of the cochlea. Acoustic stimuli were 6 kH tone bursts at 70 dB SPL.

streptomycin were dissolved in an artificial endolymph which had the following composition (mM) NaCl 1 KCl 150 The concentrations of drugs ranged from 10^{-4} to 10^{-4} g/ml The pH of the perfusate was between 7 1 and 7.3

RESULTS

Perilymphatic application of neomycin kanamycin and dihydrostreptomycin

The CM in response to 6 kHz tone bursts at 70 dB SPL was stable during the first 10 min when the scala vestibuli and tympani were perfused with artificial perilymph. As the perfusion continued the CM showed a gradual decrease in magnitude but the decrease did not exceed 30% of the initial value When the perfusion was carried out in the scala vestibuli or tympani alone the suppression of the CM was less in both cases than when the entire perilymphatic space was perfused. The AP elicited by 6 kHz tone bursts at 70 dB SPL was temporarily suppressed during the first 2 to 5 min of perfusion. The decrease of the AP ranged from 10 to 30% of the original value after which the AP gradually showed full recovery and was stable during the rest of the perfusion period The EP showed a temporary increase of 5 to 10 mV during the first 5 to 10 min of the perfusion. The EP gradually returned to the pre-perfusion level during the remaining period of the perfusion

Perfusion of the perilymphatic space with drug solutions was carried out under the same conditions as the control experiments Fig 1 shows an experiment in which neomycin at a concentration of 10⁻ g/ml was applied to the scalae vestibuli and tympani The CM did not show any appreciable suppresion during the first ten min of perfusion. Thereafter it was gradually suppressed until after 30 min it was measured to be about 50% of the initial value The changes in the AP were quite similar to those observed in the control experiments during the first half of perfusion but the AP tended to decrease gradually as the perfusion continued On the other hand the EP was stable except for an initial increase observed at the beginning of perfusion After the perfusion stopped the CM continued to decrease in amplitude while the EP remained stable during the entire period of observation

As shown in Table I the suppression of the CM and AP was related to the concentration of ototoxic antibiotics. The perfusion with oto-

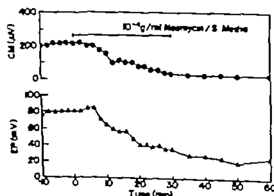


Fig 2 Effects of neomycin (10^{-4} g/ml) on the cochlear microphonic (*CM*) (top panel) and the endocochlear potential (*EP*) (bottom panel). The scala media was perfused with artificial endolymph containing neomycin (10^{-4} g/ml) for period denoted by the horizontal line. All responses were recorded from the second turn of the cochlea. Acoustic stimuli were 1 kHz tone bursts at 80 dB SPL.

Table I *Summary of effects of perilymphatic perfusion with ototoxic antibiotics on cochlear microphonics (CM) action potential (AP) and endocochlear potential (EP)*
SV scala vestibuli ST scala tympani

Drugs	Site of perfusion	Concentration (g/ml)	Cases	Loss of responses at the end of perfusion: CM >30%	CM <30%	AP >30%	AP <30%	EP >30%	EP <30%
Control	SV & ST		5	0	5	0	5	0	5
	SV		3	0	3	0	3	0	3
	ST		3	0	3	0	3	0	3
Neomycin	SV & ST	10^{-5}	3	0	3	0	3	0	3
	SV & ST	10^{-4}	3	1	2	2	1	0	3
	SV & ST	10^{-}	4	3	1	2	2	0	4
	SV	10^{-5}			0	2	0	0	2
	ST	10^{-}	2	2	0		0	0	
Kanamycin	SV & ST	10^{-5}	1	0	1	0	1	0	1
	SV & ST	10^{-4}	1	0	1	0	1	0	1
	SV & ST	10^{-}	2	1	1		0	0	2
Dihydro-streptomycin	SV & ST	10^{-4}		0	2	0		0	
	SV & ST	10^{-}		1	1	0		0	

The losses of cochlear responses at the end of perfusion were normalized by taking average pre-perfusion levels as 100% Perfusion was at a rate of 2 μl/min for a duration of 20 min Responses were recorded from the basal of the cochlea. Acoustic stimuli were 6 kHz tone bursts at 70 dB SPL.

Kelsey 1968) When both scala vestibuli and tympani were perfused a perfusion pipette was inserted into the scala tympani of the basal turn of the cochlea and a hole that served as an outlet was made in the scala vestibuli of the basal turn When the scala vestibuli or scala tympani was perfused separately a perfusion pipette was placed in a corresponding scala of the basal turn and an outlet was made at the apex of the cochlea. The rate of perfusion was about 2 μl/min and the period of perfusion was 30 min Sulfate compounds of neomycin kanamycin or dihydrostreptomycin were dissolved in an artificial perilymph which had the following composition (mM) NaCl 137 KCl 5 $CaCl_2$ 2 $NaHPO_4$ 1 $MgCl_2$ 1 $NaHCO_3$ 12 glucose 11 The concentrations of antibiotics ranged from 10^{-3} to 10^{-5} g/ml The solutions were freshly prepared before perfusions and kept at room temperature (24 C) The pH was normally about 7 5

Endolymphatic perfusion

A microperfusion technique was employed for the endolymphatic administration of drugs The technique used was essentially the same as that reported by Matsuura et al (1971*b*) A micropipette with a tip diameter of about 5 μm was connected to a polyethylene tube This system was filled with perfusate and was then connected to a polyethylene tube filled with mercury The perfusion rate was controlled by changing the height of the mercury column and was determined by measuring the movement of a small air bubble in the tube Before endolymphatic perfusion the scala vestibuli of the fourth turn was opened and Reissner s membrane was ruptured with a fine hook The perfusion pipette was then inserted into the scala media of the basal turn The rate of perfusion was 0 1 to 0 2 μl/min which could be safely used without rapid deterioration of the CM The period of perfusion was 30 min The volume of the endolymph in guinea pigs estimated by Fernández (1952) on the basis of serial histological sections of the guinea pig cochlea is about 3 μl Therefore the endolymph in the scala media could be replaced by perfusate within 15 to 30 min Sulfate compounds of neomycin kanamycin or dihydro-

lymphatic perfusion with various ototoxic antibiotics on the CM and EP In contrast to Table I the effective concentrations of antibiotics to suppress the CM or EP were lower than concentrations used in the perilymphatic perfusion. The suppression of the cochlear responses produced by endolymphatic administration was dependent on the concentrations of ototoxic antibiotics as seen in Table II Although the number of experiments with kanamycin or dihydrostreptomycin was limited there were no distinct differences among those three antibiotics in terms of ototoxicity

DISCUSSION

The present studies clearly demonstrated that the concentrations of ototoxic antibiotics necessary to suppress the CM were always lower when administered to the endolymphatic than to the perilymphatic space Matsuura et al. (1971*a*) reported that in goldfish, streptomycin and kanamycin suppressed the saccular microphonics only when administered intraluminally On the basis of our present findings it is apparent that the concentration of ototoxic antibiotics in the endolymph is a critical factor in the cause of the dysfunction of the hair cells. Morphological studies by Duvall & Wersall (1964) Hawkins & Engström (1964) and Kohonen (1965) described the first sign of hair cell degeneration during streptomycin intoxication to be a swelling and peculiar disarrangement of sensory hairs of the outer hair cells. In conjunction with these morphological findings our results imply that the primary site of action of neomycin kanamycin or dihydrostreptomycin may be the hair-bearing ends of hair cells

Stupp et al (1966, 1973) investigated the characteristic pharmacokinetics of ototoxic antibiotics administrated systemically Their quantitative bioassays indicated that high antibiotic concentrations were found in the perilymph with concentrations in the endolymph being almost as high The half life of the neomycin was about 15 hours in the inner ear fluids being 10 times longer than the half-life in the blood They concluded that the organ specificity of ototoxic antibiotics is a result of accumulation in the inner ear Balogh et al (1970) studied the distribution of tritiated dihydrostreptomycin injected intraperitoneally in guinea pigs They reported that tritiated dihydrostreptomycin reached higher concentrations in the perilymph than in the endolymph From their observations they assumed that dihydrostreptomycin probably entered the perilymphatic space from the spiral ligament and that the antibiotic reached the endolymph either directly from the stria vascularis or from the perilymphatic space However they failed to identify a preferential localization of dihydrostreptomycin Ilberg et al (1971) perfused the perilymphatic space with tritiated dihydrostreptomycin solution Their results indicated that the specific sensitivity of the hair cells to dihydrostreptomycin was not only due to the long persistence of the substance in the perilymph but also to the specific affinity of the drug to their cytoplasm. From autoradiographic studies and bioassays of distribution of antibiotics the gradual suppression of the CM and AP observed during the perfusion of the perilymphatic space with ototoxic antibiotics can be explained if these drugs reach the endolymph as suggested by Balogh et al (1970) A delay in the suppression of the CM may reflect the time which is necessary for drugs to reach a critical level before the CM is substantially suppressed

Our data indicate that local application of antibiotics suppresses the CM relatively more than the EP This is in agreement with reports by Davis et al. (1958) and Konishi et al (1967) which demonstrated small effects of the systemic injection of streptomycin and kanamycin respectively on the EP The relative insensitivity of the EP to systemic treatment with ototoxic antibiotics is quite different from the effect of ethacrynic acid which causes a rapid decline of the EP (Bosher et al 1973) Further investigation of the mechanisms underlying specific tissue affinity of the two

Table II *Summary of effects of endolymphatic perfusion with ototoxic antibiotics on cochlear microphonics (CM) and endocochlear potential (EP)*

Drugs	Concentration (g/ml)	Cases	Loss of responses at the end of perfusion			
			CM		EP	
			>50%	<50%	>50%	<50%
Control		5	1	4	0	5
Neomycin	10^{-6}	4	2		0	4
	10^{-5}	5	4	1	1	4
	10^{-4}	5	5	0	3	2
Kanamycin	10^{-6}	3	1	2	0	3
	10^{-5}	3	3	0	1	2
	10^{-4}	2	2	0	1	1
Dihydrostrepto-mycin	10^{-6}	1	0	1	0	1
	10^{-5}	1	1	0	0	1
	10^{-4}	2		0	1	1

Perfusion was at a rate of 0 1 to 0 2 µl/min Responses were recorded from the second turn of the cochlea. Losses of responses at the end of perfusion were normalized by taking average pre-perfusion values as 100% Acoustic stimuli were 1 kHz tone bursts at 80 dB SPL.

toxic antibiotics at concentrations of less than 10^{-4} g/ml did not suppress the CM or AP to a greater extent than that observed during control experiments It was also found that kanamycin dihydrostreptomycin and neomycin had similar ototoxic effects when applied to the perilymphatic space By contrast when the perfusion with neomycin at a concentration of 10^{-3} g/ml was carried out in the scala vestibuli or scala tympani alone the suppression of the CM and AP recorded at the end of perfusion was comparable to that observed during the perfusion of the entire perilymphatic space

Endolymphatic application of neomycin kanamycin and dihydrostreptomycin

When the scala media was perfused with artificial endolymph the CM in response to 1 kHz tone bursts at 80 dB SPL remained little changed during the first 5 min of perfusion It then gradually decreased in amplitude during the next ten min Usually this decrease of the CM was accompanied by an increase of the negative summating potential (SP) Thereafter the CM stabilized and the negative SP gradually decreased At the end of perfusion with control solution the CM was never suppressed by more than 50% The EP increased by 10 mV during the period when the CM was initially suppressed This rise was followed by a gradual decline until the end of perfusion The EP recorded at the end of perfusion ranged from 80 to 60% of the pre perfusion level After the end of perfusion there was no marked recovery of the CM or EP but none of the control animals showed any further decrease of these potentials

Perfusion of the scala media was carried out with drug solutions under the same conditions as the controls Fig 2 shows the suppression of the CM and EP during and after the perfusion of the scala media with neomycin at a concentration of 10^{-4} g/ml The CM showed a progressive decline commencing shortly after the perfusion started In most cases the negative SP increased in magnitude The magnitude of the CM reached the level of post mortem CM by the end of perfusion The EP was also suppressed after the initial increase and the EP observed at the end of perfusion was approximately 50% of the pre perfusion value The CM and EP did not show recovery after the perfusion ended and the EP continued to decrease gradually in magnitude

Table II summarizes the effects of endo-

lymphatic perfusion with various ototoxic antibiotics on the CM and EP In contrast to Table I the effective concentrations of antibiotics to suppress the CM or EP were lower than concentrations used in the perilymphatic perfusion. The suppression of the cochlear responses produced by endolymphatic administration was dependent on the concentrations of ototoxic antibiotics as seen in Table II Although the number of experiments with kanamycin or dihydrostreptomycin was limited, there were no distinct differences among those three antibiotics in terms of ototoxicity

DISCUSSION

The present studies clearly demonstrated that the concentrations of ototoxic antibiotics necessary to suppress the CM were always lower when administered to the endolymphatic than to the perilymphatic space Matsuura et al (1971*a*) reported that, in goldfish streptomycin and kanamycin suppressed the sacular microphonics only when administered intraluminally On the basis of our present findings it is apparent that the concentration of ototoxic antibiotics in the endolymph is a critical factor in the cause of the dysfunction of the hair cells. Morphological studies by Duvall & Wersäll (1964) Hawkins & Engström (1964) and Kohonen (1965) described the first sign of hair cell degeneration during streptomycin intoxication to be a swelling and peculiar disarrangement of sensory hairs of the outer hair cells. In conjunction with these morphological findings our results imply that the primary site of action of neomycin kanamycin or dihydrostreptomycin may be the hair-bearing ends of hair cells

Stupp et al (1966, 1973) investigated the characteristic pharmacokinetics of ototoxic antibiotics administrated systemically Their quantitative bioassays indicated that high antibiotic concentrations were found in the perilymph with concentrations in the endolymph being almost as high. The half-life of the neomycin was about 15 hours in the inner ear fluids being 10 times longer than the half life in the blood They concluded that the organ specificity of ototoxic antibiotics is a result of accumulation in the inner ear Balogh et al (1970) studied the distribution of tritiated dihydrostreptomycin injected intraperitoneally in guinea pigs They reported that tritiated dihydrostreptomycin reached higher concentrations in the perilymph than in the endolymph From their observations they assumed that dihydrostreptomycin probably entered the perilymphatic space from the spiral ligament and that the antibiotic reached the endolymph either directly from the stria vascularis or from the perilymphatic space. However they failed to identify a preferential localization of dihydrostreptomycin. Ilberg et al (1971) perfused the perilymphatic space with tritiated dihydrostreptomycin solution. Their results indicated that the specific sensitivity of the hair cells to dihydrostreptomycin was not only due to the long persistence of the substance in the perilymph but also to the specific affinity of the drug to their cytoplasm. From autoradiographic studies and bioassays of distribution of antibiotics the gradual suppression of the CM and AP observed during the perfusion of the perilymphatic space with ototoxic antibiotics can be explained if these drugs reach the endolymph as suggested by Balogh et al (1970) A delay in the suppression of the CM may reflect the time which is necessary for drugs to reach a critical level before the CM is substantially suppressed

Our data indicate that local application of antibiotics suppresses the CM relatively more than the EP This is in agreement with reports by Davis et al (1958) and Konishi et al (1967) which demonstrated small effects of the systemic injection of streptomycin and kanamycin respectively on the EP The relative insensitivity of the EP to systemic treatment with ototoxic antibiotics is quite different from the effect of ethacrynic acid which causes a rapid decline of the EP (Bosher et al 1973) Further investigation of the mechanisms underlying specific tissue affinity of the two

groups of ototoxic drugs is necessary before the mode of action of these drugs can be elucidated

ZUSAMMENFASSUNG

Es wurde in Meerschweinchen bei lokaler Verabreichung mittels Durchströmung der Einfluß von Neomycin Kanamycin bzw Dihydrostreptomycin auf die Cochlear mikrofonie das Wirkungspotential des Gehörnerven sowie das Endocochlearpotential untersucht. Die Unterdrückung der cochlearen Reaktionen war besonders merklich wenn diese Mittel der Endolymphe weniger spürbar wenn sie der Perilymphe zugeführt wurden. Der Wirkungsmechanismus der Antibiotika auf die Haarzellen des Cortischen Organs wird besprochen

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SOME VASCULAR EFFECTS OF NOISE EXPOSURE IN THE CHINCHILLA COCHLEA

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Abstract Chinchillas were exposed to pink noise at levels ranging from 110 dB (for 8 hours) to 125 dB (for 15 min). After 3-week survival period the animals were killed and cochlear tissues were prepared using soft-surface preparation technique To evaluate discrete changes of the cochlear vasculature and minimize bias with respect to what was judged pathological, method was used hereby experimental and control animals were mixed and randomly assessed without prior knowledge of the group to hich the specimen belonged. The results were analysed by computer Cochlear hair cell damage was slight. Statistically significant differences in the vasculature between noise-exposed and control animals were few For example in some cochlear vessels in the experimental animals, red blood cells were found to be less frequent, other vessels showed an increase or decrease in endothelial or periendothelial cells A more prominent difference between control and noise-exposed animals was an increased variability in several of the adopted vascular parameters, thus disturbing the normal regular patter and appearance of the vascular bed. This was especially evident with respect to the frequency and spacing of red blood cells in the vessel lumen Contrarily some parameters previously considered to be typical examples of scular degeneration such as a ascular channels and perivascular spaces were found equally often in noise exposed and control animals Some possible explanations regarding the observed vascular changes are discussed

It has been theorized that noise-induced changes in the cochlear vasculature precede and perhaps contribute to hair cell damage produced by acoustic trauma (Lawrence et al 1967 Spoendlin 1971 Kellerhals 1972*a b* ipscomb & Roettger 1973) In studying the lood supply of the cochlea quite often the naterial has been limited in some way such hat relatively few vessels and/or vascular parameters have been examined. In micro-circulation experiments for example only relatively few vessels in the external wall or spiral lamina are accessible for study Further limitations are often made by the experimenter Thus only those vessels thought to have an immediate and/or important effect on the sensory cells of the organ of Corti are studied Most typically researchers describe the condition of the vessel of the basilar membrane (the outer spiral vessel) (Lawrence et al 1967 Lipscomb & Roettger 1973) and of the capillary network of the stria vascularis (Lawrence 1972 Duvall et al 1974)

In addition to limiting the number of vessels to be observed and analysed researchers also often restrict the area of measurement and investigation The condition of the vessel of the basilar membrane and/or stria vascularis then is not studied in the entire cochlea but is most typically studied in the area of the cochlea where greatest hair cell damage occurs

It is often difficult to determine which vascular findings constitute pathology in the cochlear blood supply Gross vascular changes such as greatly constricted collapsed extremely dilated or completely degenerated vessels are clearly seen. However more minute changes may be overlooked At the light microscopic level two factors in

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Table I *Regularly occurring vessels of the chinchilla cochlea*

EXTERNAL WALL
Scala vestibuli
Radiating arterioles
Collecting venules
The vessel at the vestibular membrane
Scala media
Arteriovenous anastomoses
Stria vascularis
The vessel of the spiral prominence
Scala tympani
The venules at the basilar membrane
Collecting venules
SPIRAL LAMINA
Radiating arterioles & collecting venules
The limbus vessels
The vessel of the basilar membrane
The vessel of the tympanic lip

particular have been examined for indications of vascular pathology One is the frequency or so-called density of red blood corpuscles in the various vessels and the other is the presence and condition of various perivascular structures (Hawkins 1967 1971 Lawrence et al 1967 Lipscomb & Roettger 1973)

In the following study all the regularly occurring cochlear vessels in all cochlear turns of noise-exposed and control animals were examined Ten vascular parameters each considered to reflect in some way the condition of the cochlear blood supply were examined

MATERIAL AND METHODS

Seven chinchillas were bilaterally exposed once to pink noise of varying intensity and duration such that noise levels increased by 3 dB from 110 dB (for 8 hours) to 125 dB (for $^1/_4$ hour) The time factor decreased by one-half for each 3 dB increase in noise intensity Realistic speaker systems driven by a Mac Intosh 40 power amplifier were used to deliver the noise which was produced by a General Radio random noise generator and filtered by a General Radio universal filter before amplification The noise had a rolloff of approximately 3 dB/octave When being noise-exposed each animal was kept in a restraining cage with its head at zero degrees azimuth approximately 4 inches from the plane of the speaker Only one animal was exposed at each level from 110 dB to 122 dB while 2 animals were exposed at 125 dB Four animals served as controls

After a 3-week survival period the animals were killed without anesthesia by decapitation The tissues were prepared using a so surface preparation technique previously described (Axelsson et al 1974 1975) with the exception of the injection of contrast in vessels After immediate removal of the temporal bones by a ventral approach the bulla was opened and the stapes removed A fixative solution of paraformaldehyde–glutaraldehyde was injected slowly through the round window After remaining in the fixative for 8 hours the cochlea was transferred to 8 EDTA for decalcification This solution was changed once every 12 hours until decalcification was complete Dissection began with mid or para-modiolar section of the cochlea after which both halves were examined for gross pathology Specimens were then counter stained with Veronal-buffered osmic acid (0.5%) for approximately 10 minutes dehydrated in successive stages of increasing concentrated alcohol immersed in glycerin and stored

One longitudinal half from each cochlea was further dissected and all structures in each of the three turns was placed on a randomly numbered slide Using phase- or interference-contrast microscopy the observers examined all experimental and control tissues without having prior knowledge of the experimental group to which they belonged All the regularly occurring cochlear vessels shown in Table I were examined for evidence of pathologic changes Some of the changes measured as well as our rationale for choosing these particular parameters have previously been di

Table II *Vascular parameters*

Frequency and spacing of red blood corpuscles
Red blood corpuscle orientation in vessel lumen
Frequency of perivascular spaces
Frequency of vascular channels
Frequency of pericytes
Size of pericytes
Pericyte influence on vessel lumen
Frequency of endothelial cell nuclei
Size of endothelial cell nuclei
Endothelial cell nucleus influence on vessel lumen

cussed (Axelsson & Vertes 1977) In addition to evaluating the number of red blood corpuscles and the frequency size and influence of endothelial cell nuclei (hereafter referred to as endothelial cells) and pericytes all of the parameters shown in Table II were also examined in each vessel In essence this means that in each cochlea all 10 vascular parameters were read in each of the 12 cochlear vessels in the spiral lamina and external wall in all cochlear turns This amounts to over 700 observations per animal or nearly 8 000 total observations Missing hair cells and hair cells replaced by the collapsed phalangeal processes of the Deiter cells were noted as "damaged" on the cytocochleogram (Engström et al 1966) Representative findings were documented with phase contrast photomicrography

In view of the large amount of data generated by this method it was necessary to devise a computer technique by which to analyse the data With respect to the vasculature we were particularly interested in determining if differences existed between ears among the cochlear turns and among vessel groups, i.e arterioles vs capillaries vs venules or spiral lamina vessels vs external wall vessels All data regarding both hair cell and vascular pathology were evaluated by means of computer analysis Differences between means were tested using the Mann-Whitney test (Seigel 1956) a 0 05 level of significance was adopted.

RESULTS

General findings

Gross examination of the cochlea after mid modiolar sectioning showed that there was often a small hemorrhage in the area inside the round window This finding was equally frequent in noise-exposed animals and controls The condition of the vestibular membrane was also observed and it appeared distended or normal in all cochleas again there seemed to be no difference between noise and control animals At this level of observation, gross alterations were obvious neither in the sensorineural epithelium nor in the cochlear vasculature

Sensorineural epithelium

The levels and durations of the noise exposure used in this study were apparently insufficient to cause any significant degree of inner or outer hair cell pathology There were no gross differences between the animals having different noise exposures The 2 animals which sustained greatest injury (10% and 7% total cell loss) had apparent hair cell damage at one ear only In general in both noise and control animals the hair cell loss which did occur was not localized in any one specific area but was spread throughout the entire cochlea. Hair cell damage was greatest in the third row of outer hair cells and decreased toward the inner hair cells in all animals

Cochlear vasculature

The condition of the vasculature at both the right and left ears of each animal was essentially similar Unlike the sensory cell findings differences in the cochlear vasculature between noise and control animals were found to increase from base to apex. When considering all vascular parameters as a group noise and control animals were similar in turn one However differences between experimental and control animals increased in more apical turns and were greater in turn 3 than in turn 2

Table III *Mean values significantly different in noise than controls*

Red blood cells *less* frequent and spaced further apart in
Radiating arterioles & collecting venules spiral lamina
Limbus vessels
Red blood cell orientation in lumen different in
Vessel of the basilar membrane (oblique vs longitudinal)
Endothelial cell nuclei *more* frequent in
Vessel of the spiral prominence
Endothelial cell nuclei *less* frequent in
Vessel of the basilar membrane
Pericytes *more* frequent in
Limbus vessels
Vessel of the tympanic lip

Data analysis showed that the cochlear blood vessels seemed to form two distinct groups in terms of commonality of the vascular parameters we observed This was true in spite of the fact that each group is composed of arterioles capillaries and venules One group was the external wall vessels and the other was the spiral lamina vessels Noise induced vascular changes occurred more frequently in spiral lamina vessels than in external wall vessels

Arterioles and venules were found to differ along several parameters in the control animals Noise exposure however seemed to influence the arterioles and venules so that they became more similar especially with respect to the manner in which the red blood cells were oriented within the lumen and to the frequency of endothelial cells and pericytes Changes as a result of noise exposure more often occurred in venules than arterioles in this respect

Statistically significant vascular changes may be divided into two types One type involves a mean change in how often a parameter occurred and the other type includes variability changes Changes in the mean values of the vascular parameters and the vessel in which they occur are shown in Table III Those factors which were more variable in noise than control animals and the vessels in which they occur are shown in Table IV Some representative findings are also shown in Figs 1 and 2 Instances in which the vascular parameters were significantly less variable in noise-exposed than control animals did not occur

In addition to the computer analysed data collected on the bascular parameters shown in Table II any extraordinary or unusual changes in the vessels were also noted The collection of these comments indicated that the following factors were more common in noise-exposed animals than in controls More frequent deposits of an osmiophilic material as seen in Fig 3 were found surrounding the vessels in the region above the attachment of the vestibular membrane In 2 animals one exposed to 113 dB for 4 hours and one to 11[illegible] dB for 2 hours sludging was seen in the central spiral lamina vessels and once in the limbus vessels However in 2 other animals one exposed to 122 dB for 30 min and one to 1[illegible] dB for 15 min the central spiral lamina vessels were found to have few red blood corpuscles The vessel of the basilar membrane was usually empty of red blood corpuscles however in the experimental animal exposed to 122 d[illegible]

Table IV *Variability significantly different in noise than controls*

Frequency and spacing of red blood cells more variable in
Collecting venules scala vestibuli external wall
Arteriovenous anastomoses
Radiating arterioles & collecting venules-spiral lamina
Limbus vessels
Vessel of the tympanic lip
Red blood cell orientation in vessel lumen more variable in
Collecting venules scala tympani-external wall
Endothelial cell nuclei influence on vessel lumen more variable i
Collecting venules scala tympani-external wall
Vessel of the tympanic lip
Pericyte size more variable in
Vessel of the basilar membrane

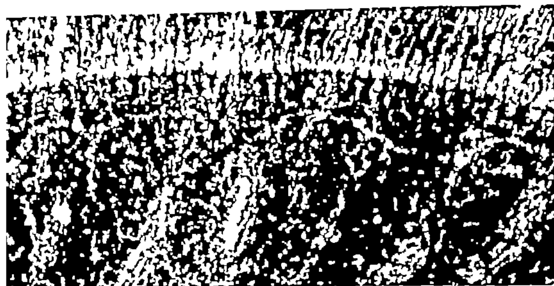


Fig. 1. Chinchilla spiral limbus, 2nd turn. In some vessels of noise-exposed animals red blood cells were less frequent and spaced further apart. In this view limbus vessels (*LVS*) appear empty.

for 30 min the vessel of the basilar membrane in one area was found to be quite thick and packed with red blood cells. Finally the surface epithelium of the stria vascularis was often degenerated.

DISCUSSION

The aim of the present study was to make a thorough investigation of the effects of noise on the chinchilla cochlear vessels using computer analysis so as to identify discrete dif



Fig. 2. Chinchilla spiral lamina, apical turn. In noise-exposed animals the frequency of pericytes increased in some vessels. Three distinctive pericytes (arrows) can be seen in this nearly empty vessel of the tympanic lip (*VSTL*).

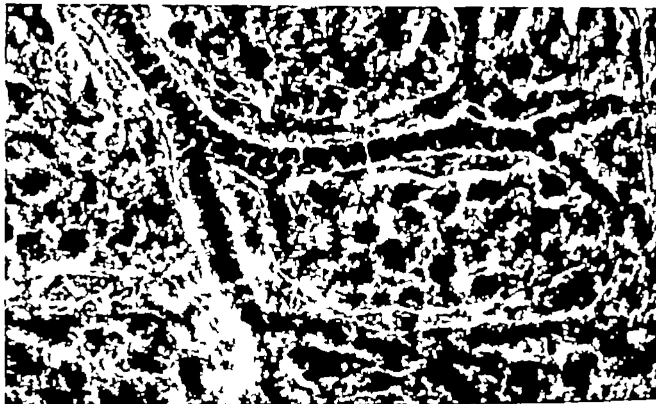


Fig 3 Chinchilla external wall 2nd turn In noise exposed animals deposits (*arrows*) were more frequently seen surrounding vessels close to the attachment of the vestibular membrane *VSVM*=vessel at the vestibular membrane

ferences between experimental and control animals Originally it was our intention to make a completely numerical analysis thereby giving, for example the number of red blood cells per vessel length or the diameter of vessels in microns These quantitative" measurements were going to replace our presently-used qualitative ones Our present method involves assigning a subjective value to each parameter based on observations of vessels made within the whole microscopic field This is done without knowing whether the specimen belongs to the control or experimental group After consulting with statisticians however it was concluded that our present method is a quite satisfactory manner of evaluating vascular change

Another purpose of our study was to obtain information about how our present technique might be modified to make it faster and easier The results of this study have been quite helpful in this respect We were able to determine for example that under the noise conditions used the vasculature in the chinchilla is similarly affected at both ears Contrarily however we found that noise at least of the sort used in this study affected the cochlear turns differently thus it is necessary to continue making our vascular readings throughout the cochlea

The levels and durations of the basically low frequency pink noise used in this study produced very limited hair cell damage No gross differences in hair cell damage were observable between animals exposed to different noise levels or durations Contrarily also in the chinchilla similarly long and intense exposures to noises with most of their acoustic energy concentrated in the higher frequencies are known to cause significant hair cell pathology (Ward & Duvall 1971 Eldredge 1973, Clark et al 1974 Hunter Duvar & Bredberg 1974 Vertes & Nábělek 1977) Based on head and pinna diffraction and ear canal resonances in the chinchilla however it would be expected that to be equally damaging to the

ensory hair cells low frequency noise must e increased either in intensity or duration Eldredge 1973) Whether or not the vascular pathology observed at these exposures contributed to the hair cell loss which did occur s unknown at this time

A small perilymphatic hemorrhage present on the inside of the round window was a common finding in this study Similar observations have repeatedly been made in control animals of different species as well as in investigations of barotrauma, mechanical lesions and noise exposure (Axelsson & Hallén 1973 Lamkin et al. 1975 Axelsson et al. 1977) Whether these hemorrhages result from our termination technique or represent an in vivo condition in these animals is not yet possible to determine Other authors have often published photomicrographs showing hemorrhages in the intracochlear lymphs but have seldom made any reference to either their presence or possible cause

As stated in the introduction certain limitations are frequently made by experimenters studying the cochlear vasculature Thus only a few of the regularly occurring cochlear vessels are studied the two most common of which are the vessel of the basilar membrane and the capillaries of the stria vascularis By evaluating isolated vessels the importance of the vascular bed as a single functional circulatory unit with different types of vessels influencing each other will be missed Further *the results of this study indicate that by* so limiting the vessels to be studied, some important vessels will be overlooked More importantly as seen in Tables III and IV these include the vessel of the tympanic lip the limbus vessels and the collecting venules of the scala tympani of the external wall

The second limitation regarded the location of measurement and investigation, i e the vasculature is often studied in the vicinity of greatest hair cell pathology Our results showed that with this particular noise what hair cell damage did occur was spread throughout the cochlea. Vascular pathology however increased from base to apex The lack of agreement between the location of sensory hair cell and vascular pathology has been noted previously (Axelsson 1968 Bohne 1976 Fried et al 1976) It would seem then that until a consistent correlation if one exists can be found between the location of noise induced hair cell loss and vascular pathology the vasculature must be studied throughout the entire cochlea.

The third limitation mentioned in the introduction dealt with factors thought to be indicators of vascular pathology As mentioned the frequency or density of red blood corpuscles in the various vessels is most typically studied. Often in conjunction with this the frequency of endothelial cells and pericytes and their size and influence on the vessel lumen are examined Our findings confirm the importance of these factors in that both the mean frequency and spacing and the variability of the frequency and spacing of red blood cells was found to differ between noise and control animals in quite a few of the cochlear vessels (see Figs 1 and 2) In addition one or more of the dimensions regarding the endothelial cells and pericytes as shown in Tables III and IV were also found to be significantly different between noise-exposed animals and controls Contrarily the existence of perivascular spaces and avascular channels which are also quite frequently mentioned as degenerative phenomena contributed little to the differentiation of experimental from control animals In the chinchilla then these two parameters would seem to be irrelevent at least with the levels and durations of the particular noise exposure used in this study

The specific results of this study are not very easily explained. It is difficult to understand how a short-term relatively innocuous noise which caused an average of 3% total hair cell damage is still able to cause temporary or perhaps permanent vascular pathology The decreased mean frequency and/or increased variability of the frequency of red blood cells after noise would seem to reflect

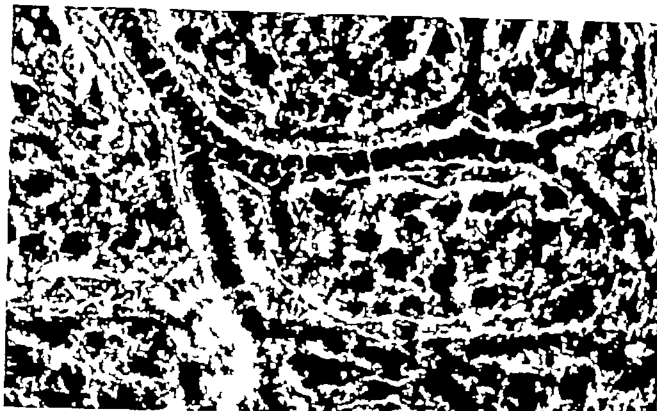


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an increase in the blood flow rate present at death 3 weeks post-exposure An increased resistance caused by narrowed vessels could be responsible for such changes in blood flow It is known that increases in transmural pressure lead to changes in vessel wall thickness (Folkow 1956 Folkow et al 1958 Short & Thomson 1959 Short 1966) Perlman & Kimura (1962) found that while low level noise causes no visible change in the blood flow rate or in the vessel diameter there was a tendency for the flow rate to increase with increasing noise level In the vessels examined flow rate returned to normal 30 minutes after the termination of the exposure The authors noted only occasional vessel dilation and narrowing of vessels was uncommon Reduction in blood flow rate was not observed Changes in microcirculation can be understood to occur *during* acoustic overstimulation that they can still or again be present 3 weeks following such short-term and mild noise exposures as used in this study is surprising Nevertheless the possibility cannot be rejected that even mild noise exposures can cause long-term or perhaps even permanent structural alterations in the cochlear vasculature

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ZUSAMMENFASSUNG

Chinchillas wurden mäßigem Lärm ausgesetzt Drei Wochen später wurden die Tiere getötet und die Cochlea-Gewebe mit einer Häutchen-Präparatmethode untersucht. Um kleine Veränderungen in den Gefäßen der Cochlea zu beurteilen und subjektive Beeinflussung der Grenzen pathologische Veränderungen zu vermeiden wurden Experiment und Kontrolltiere vermischt und randomisiert studiert ohne daß der Untersucher wußt zu welche Gruppe das studierte Präparat gehörte Die Befunde wurden mit Computer analysiert Schäden der Haarzellen waren gering Ein statistisch signifikanter Unterschied der Gefäße der lärmexponierten und der Kontrolltiere war auch ungewöhnlich In einigen Gefäßen der Cochlea in den lärmexponierten Tieren waren rote Blutkörperchen seltener als in den Kontrolltieren In anderen Gefäßen konnte man eine Zunahme od Abnahme der Anzahl von endotelialen und periendotelialen Zellen beobachten

Ein deutlicherer Unterschied zwischen Kontroll- und lärmexponierten Tieren fand sich in Form einer erhöhten Variabilität in mehreren der benutzten Gefäßparameter Dieses verminderte das normale regelmäßige Bild d Cochlea-Gefäße Dieser Befund war besonders gewöhnlich betreffs Vorkommen und Dichte der roten Blutkörperchen im Gefäßlumen Im Gegensatz dazu wurd andere Befunde die man früher als typische Beispie einer Gefäßdegeneration angesehen hat z. B ...ulläre Kanäle und perivaskuläre Räume ebenso oft lärmexponierten als in Kontrolltieren gefunden

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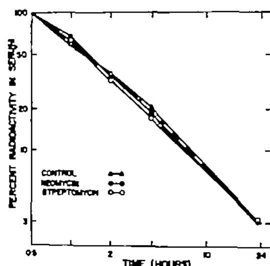


Fig. 1. Retention of radioactive calcium in serum. ^{45}Ca in serum was measured at 0.5, 1, 2, 4 and 24 hours. Average points are shown for 15 control animals, 15 streptomycin-treated animals, and 13 neomycin-treated animals.

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For scintillation counting, otolithic membranes were air-dried of the ethanol and dissolved in 1 N HCl. Upon evaporation of the HCl, the scintillant was added and the radioactivity determined. Otic bone and femur chips were decalcified with concentrated formic acid for 3 days, and an aliquot was drawn for liquid scintillation counting. Usually pairs of saccular or utricular otolithic membranes were examined together. Weights of the otolithic membranes were not obtained and the uptake of $^{45}CaCl_2$ was determined for each pair of membranes.

RESULTS

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This part of the study will be reported in detail elsewhere. In most animals the otoconia covered the entire macula as described and pictured in the literature (Engström et al. 1966, Lindeman 1969, Johnsson & Hawkins 1967, Hawkins & Johnsson 1975, Preston et al. 1975). Abnormal appearing otolithic membranes were present in approximately one third of the animals. These guinea pigs had a markedly reduced number of otoconia in the saccule. The utricle had few or no otoconia above the striola line, giving the appearance of a curved groove or valley in the otoconial layer rather the ridge commonly seen and referred to as the snowdrift line (Engström et al. 1966). The saccular and utricular findings always occurred hand in hand, were bilateral and more or less symmetrical. This abnormality was not caused by drug treatment, since it was found to roughly the same extent in drug-treated as in untreated control animals. Identification of these animals during dissection was both easy and necessary for the proper evaluation of the results. The markedly reduced and variable mass of otoconia rendered these particular guinea pigs unsuitable for the present incorporation study and they are referred to as "defective" in the text.

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Acta Otolaryngol 88 56–60 1979

INCORPORATION OF RADIOACTIVE CALCIUM INTO OTOLITHIC MEMBRANES OF THE GUINEA PIG AFTER AMINOGLYCOSIDE TREATMENT

Iris Mechigian Robert E Preston Lars-Göran Johnsson and Jochen Schacht

From the Kresge Hearing Research Institute and Department of Otorhinolaryngology University of Michigan Medical School Ann Arbor USA

(Received October 23 1978)

Abstract The influence of neomycin and streptomycin on the calcium metabolism of the otolithic membranes was investigated in the guinea pig. After chronic treatment with either drug animals were injected intraperitoneally with radioactive calcium Retention of calcium in the serum was unaffected by drug treatment as was the incorporation of radioactivity into bone (femur and otic capsule) Both drugs inhibited the calcium uptake into saccular and utricular otolithic membranes by 30 to 40%

We have previously demonstrated uptake of radioactive calcium into both saccular and utricular otoconia in gerbils (Preston et al 1975) Such uptake could reflect growth of existing crystals neogenesis of otoconia or an exchange of Ca^{++} between endolymph and otoconial $CaCO_3$ The reduction in size and number of otoconia with aging in man (Johnson & Hawkins 1972 Ross et al 1976) is in accord with our belief that otoconia are exposed to attrition and depend on an active metabolism for their maintenance

It is not known which structures are involved in the formation and maintenance of the otoconia but it is tempting to speculate that the neuroepithelium underlying the otolithic membrane participates in this process We therefore studied the effect of aminoglycosides on calcium incorporation because of their well known ototoxic action on the neuroepithelium Two drugs known to produce different patterns of damage were chosen. streptomycin which causes injury mainly to crista ampullaris and the otolithic organs and neomycin which is primarily toxic to the cochlea but also affects the saccular neuroepithelium (Hawkins 1976)

Preliminary experiments had demonstrated that the gerbil was highly resistant to the ototoxicity of neomycin and streptomycin In the guinea pig the effects of aminoglycosides have been well established and therefore these animals were used in this study

METHODS

Forty-eight albino guinea pigs of either sex (250–300 g) were divided into three groups and subjected to the following treatments

(1) streptomycin sulfate in saline (200 mg streptomycin base/kg body weight) injected subcutaneously daily for 20–22 days

(2) neomycin sulfate (100 mg neomycin base/kg body weight) injected subcutaneously daily for 20–22 days

(3) saline (1 ml/kg body weight) injected subcutaneously daily for 20–22 days

Twenty four hours after the last injection each animal was given $^{45}CaCl_2$ in saline (4 μCi/g body weight) intraperitoneally

Blood samples were obtained at 1/2 hour 1 hour 2 hours 4 hours and 24 hours after isotope injection Each animal was lightly anesthetized with ether and 0 5–1 0 ml of blood was drawn with a pipette from the orbital venous plexus The samples were then refrigerated and centrifuged to obtain serum for scintillation counting.

Twenty four hours after isotope injection the animals were killed by decapitation The bullae were immediately removed and the

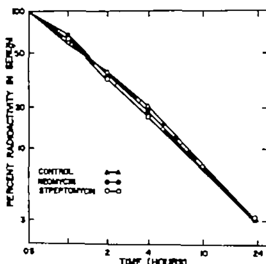


Fig. 1. Retention of radioactive calcium in serum. ⁴⁵Ca in serum was measured at 0.5, 1, 2, 4 and 24 hours. Average points are shown for 15 control animals, 15 streptomycin-treated animals, and 13 neomycin-treated animals.

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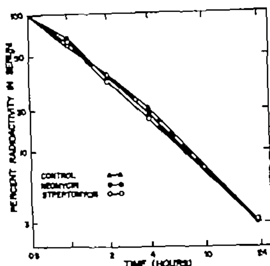


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Table I *Calcium incorporation into bone expressed as μmoles Ca incorporated/gram*

Treatment	Femur (μmoles)	Otic capsule (μmoles)
None (controls)	6±10 (15)	78±36 (14)
Streptomycin	23±7 (15)	78±41 (1)
Neomycin	25±8 (13)	94±68 (10)

Incorporation was calculated from radioactivity in bone samples and serum radioactivity over 24 hours (see text) Numbers are means ± S D with numbers of animals in parentheses Differences are not s gnificant by *t* test.

line These lesions were roundish and varied in size If large enough the otoconia above the epithelial defect were missing These membranes appeared totally different from the membranes found in the defective animals Neomycin treated animals showed no visible changes of macular morphology

Retention of ^{45}*Ca in serum* Values for serum radioactivity plotted against time gave a straight line on a double logarithmic plot A regression analysis of this curve was carried out for each animal and data were analyzed further only if a good fit of the regression curve to the data points was obtained ($r^2 \geq 0\ 9$) Five out of 48 animals did not fit this criterion

The average slope of these curves (Fig 1) for the normal animals was −90±10 S D (log cpm ^{45}Ca/log time) This meant that approximately 5% of the radioactivity found 1/2 hour after injection was still present after 24 hours There were no statistically significant differences between control animals and those receiving either neomycin or streptomycin

^{45}*Ca incorporation into otolithic membranes and bone* For each of the animals ^{45}Ca in the serum was integrated for the 24 hour period from isotope injection to sacrifice This value was the basis to which calcium incorporation into otolithic membranes and bone was normalized

Incorporation into neither femur nor otic capsule (Table I) was significantly affected by treatment of the animals with antibiotics

In contrast calcium incorporation into otolithic membranes was influenced by both neomycin and streptomycin (Table II) There was a significant 30–40% decrease in the rate of incorporation into both the saccular and utricular otolithic membranes

No attempts were made to determine whether the guinea pigs with defective" otolithic membranes showed less uptake in relation to the total mass of otoconia compared with the animals with a normal complement of otoconia We can only conclude that the absolute incorporation per sample was much smaller in these animals

DISCUSSION

The present study demonstrates an active incorporation of serum calcium into the otolithic membranes in the adult guinea pig. The significant finding is that neomycin and streptomycin specifically interfere with calcium uptake into the otolithic membranes No effects were seen on the retention of calcium in the serum or on the uptake into femur and otic capsule

The ototoxic drugs used in this experiment caused the expected degeneration of the macular neuroepithelium with its supporting elements whereby streptomycin caused more serious degeneration in the macula utriculi

Table II *Calcium incorporation into otolithic membranes expressed as pmoles Ca incorporated/pair*

Treatment	Saccule (pmoles)	Utricle (pmoles)
None (Normal controls)	11 ± 6 (9)	169±36 (9)
Streptomycin	81±35 (13)*	99±53 (15)*
Neomycin	76±15 (8)*	90±3 (11)**
None ("defective controls)	4 ±18 (7)*	83±48 (6)*

Incorporation was calculated from radioacti ity in a pair of otolithic membranes and from serum radioactivity over 4 hr (see t xt) N mbers are means ± S D with numbers of animals in parentheses Significance of difference from normal controls was analysed by one way ANOVA
$0\ 02 > p > 0\ 01$ $p < 0\ 01$

than neomycin did in the saccular macula. Streptomycin also had a more specific effect on the type I sensory cells. No visible effects of the neomycin on the macula utriculi was observed. In some of the streptomycin-treated animals, there was a circumscribed loss of otoconia which in all likelihood affected the amount of calcium uptake. The fact, however, that streptomycin also caused a decreased uptake in saccular membranes which displayed no visible loss of otoconia suggests that the total mass of otoconia was only one of several determining factors in this experiment.

It seems surprising that the two drugs which caused different degrees of morphological damage to the maculae apparently decreased the calcium uptake in both the saccule and utricle to more or less the same percentage. It seems likely that the observed uptake mainly reflects incorporation into the otoconial $CaCO_3$ which represents the largest part of the mass in the specimens. Since the samples also included the membranous portion (Johnsson & Hawkins, 1967), some calcium incorporation could have occurred into this layer of the membrane or into the organic substance holding the crystals together.

The mode of action of ototoxic drugs is complex. In addition to acting on the sensory cells, they also alter the so-called "dark cells" in the labyrinth (Hawkins & Preston, 1975) and thus probably affect the homeostasis of the inner ear fluids which in turn may cause sensory cell loss. In our experiments, the streptomycin dosage was sufficient to have damaged the "dark cells" in the wall of the utricle. The possible function of the dark cells in the maintenance of the otoconia remains enigmatic (Lim, 1973) and it is impossible to say if or to what extent such changes affected the calcium incorporation.

Recently, Schacht and collaborators (Schacht, 1976; Schacht et al., 1977) have proposed a biochemical explanation for aminoglycoside-induced cochlear toxicity. Part of the suggested mechanism assumes a displacement of calcium from binding sites in the cell membranes by the drugs. The basis for the decreased uptake of calcium into otolithic membranes in the presence of drugs remains uncertain. The cause may be manifold and as complex as the action of the drugs themselves. In order to account for the lack of effect on the bone, one must invoke a site-specific mechanism in the vestibular system. This may be a specific site for calcium binding which is absent from other tissues in analogy to the proposed mechanism of the auditory toxicity. On the other hand, an elevated and persistent concentration of aminoglycosides in the inner ear fluids may be a contributing factor. Damage to the neuroepithelium and notably the supporting elements could be directly responsible for a decreased renewal of the otoconia. Yet the fact that neomycin decreased the calcium uptake in the utricle without visibly involving its neuroepithelium makes the hypothesis appear oversimplified.

It is evident from these experiments that the incorporation of serum calcium into the otolithic membranes can be influenced by ototoxic drugs. It is not clear how the calcium turnover of the otoconia relates to the systemic calcium metabolism and notably that of the bone which lies in such a close proximity to the maculae. Further studies of the metabolism of otoconia under the influence of other ototoxic drugs, calcium regulating hormones, or weightlessness are needed.

ZUSAMMENFASSUNG

Der Einfluß von Neomycin und Streptomycin auf den Kalzium-Metabolismus der otolithischen Membranen wurde in Meerschweinchen untersucht. Nach chronischer Gabe der Antibiotika wurden die Tiere intraperitoneal mit radioaktivem Kalzium injiziert. Drogenbehandlung hatte keinen Einfluß auf die Retention der Radioaktivität im Serum und den Kalziumeinbau in Knochen (Femur und Labyrinthkapsel). Beide Antibiotika erniedrigten die Einbaurate in die otolithischen Membranen der Macula utriculi und sacculi um 30 bis 40 %.

ACKNOWLEDGEMENT

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MELANIN CAPACITY TO ACCUMULATE DRUGS IN THE INTERNAL EAR

A Study on Lidocaine, Bupivacaine and Chlorpromazine

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Abstract. The distribution and retention of labelled lidocaine, bupivacaine and chlorpromazine to melanin in the internal ear after intravenous and intraperitoneal injection were examined by whole-body autoradiography. Both young pigmented hooded rats and albino rats were studied. In the pigmented rats chlorpromazine showed the greatest accumulation, which was more pronounced in the cochlea than in the vestibular portion. The other two substances were evenly distributed in the internal ear. After a single injection of chlorpromazine and of bupivacaine these substances were still bound to the melanin of the internal ear after 14 days, which was the longest survival time. Lidocaine, on the other hand, had disappeared after only 4 days. Strong uptake and retention of the three substances were observed in the eyes of pigmented animals. In albino animals there was very weak, transient uptake in the internal ear of chlorpromazine and bupivacaine but not of lidocaine. In studies *in vitro* on isolated bovine eye melanin there was considerably greater adsorption of chlorpromazine than of lidocaine and bupivacaine. An uptake was noted in the human eye in experiments *in vitro*. Clinical tests revealed no acute or late damage to hearing or sight after large doses of lidocaine. The participation of melanin in different basal labyrinthine functions such as the energy transfer mechanism and the sound protective mechanism is discussed in the light of the results obtained. Further, the theory is put forward that the melanin affinity of certain substances can be of both therapeutic and ototoxic importance.

The capacity of melanin to accumulate chloroquine has been well documented (Potts, 1964; Ullberg et al., 1970; Lindquist & Ullberg, 1972; Lindquist, 1973; Dencker & Lindquist, 1975). Prolonged high-dose therapy is known to cause damage to the eyes (Meier-Ruge, 1973) as well as to the internal ears (Hart & Naunton, 1964; Toone et al., 1965). The strongest affinity for melanin has been noted for polycyclic amines. Besides chloroquine this category also includes antibiotics of the amino-glycoside type and phenothiazine derivatives.

The uptake of drugs by melanin can be studied *in vitro* as well as *in vivo*. Differences in the uptake under these two conditions have been observed (Lindquist, 1973). *In vitro* investigations of the uptake can be made according to the method of Potts (1964), using isolated beef eye melanin granules. With this technique (Lindquist, 1973) a very high uptake of kanamycin (89%), streptomycin (60%) and chloroquine (85%) has been found. Low uptake has been reported for lidocaine (Englesson et al., 1976) and no uptake of salicylic acid (Lindquist, 1973).

In vivo investigations based on whole-body autoradiography as described by Ullberg (1954, 1977) have revealed substantial accumulation of chloroquine and chlorpromazine in the melanin-bearing tissues in the eye, skin and hair-follicles (Lindquist & Ullberg, 1972). The same authors also observed for the first time an accumulation of chloroquine in the internal ear of pigmented animals. The detailed distribution of labelled chloroquine in the labyrinth was described shortly afterwards (Dencker & Lindquist, 1975).

The aim of this study is twofold: (*a*) to study acute and late effects upon hearing and vision in man of large doses of lidocaine which according to our previous findings ac-

cumulates in the melanin in the internal ear and (*b*) to investigate autoradiographically the adsorption of bupivacaine and chlorpromazine to melanin in the internal ear both *in vivo* and *in vitro* Bupivacaine was selected because it is a long-acting local anaesthetic with a potential mitigating effect upon tinnitus Chlorpromazine was investigated for two reasons Firstly because this substance has a well documented local anaesthetic effect Secondly the substance being a phenothiazine derivative it is richly accumulated by the melanin in the eye and the skin (Lindquist & Ullberg 1972) so that a substantial uptake in the internal ear could be expected

Distribution of pigment in the internal ear

Areas of pigmentation are easily seen in the cochlea and the vestibular part of the labyrinth of pigmented animals and man but they appear to be absent from the labyrinth of albinos (Wolff 1931) The pigment in the internal ear is commonly considered to be melanin (Beck 1961) It appears in two different forms either freely as small round clusters of 7–10 μm or included in melanocytes (Savin 1965 Hilding & Ginzburg 1977) These melanocytes can have close contacts with capillaries and are found profusely in well vascularized areas of apparent secretory or metabolic importance

In man the pigment cells are scattered throughout the internal ear except for the semicircular canals Pigment cells are most common in the cochlea their density being greatest on the bony wall of the modiolus and the osseous spiral lamina (La Ferriere et al 1974) In the modiolus they lie in the delicate connective tissue in the interstices of the cribriform area and along the connective tissue forming the proximal wall of the scala (Wolff 1931) They also occur in Reissner s membrane The pigment cells in the stria vascularis are more abundant than in the spiral ligament (Savin 1965) In the stria vascularis the pigment cells are identical with the intermediate cells which are therefore considered to be melanocytes derived from the neural crest (Hilding & Ginzburg 1977)

In the vestibular part of the labyrinth in man pigments are found in the walls of the utricle and saccule and in the crus commune and the ampullae but not in the semicircular canals. The pigments can be seen on both sides of the cristae (Sieber & Schmidt 1962) not inside the cells but dispersed as fine granulations or clusters (Savin 1965) Pigment cells in close relation to minor blood vessels are observed in the ampullar endings (Savin 1965)

In contrast to man no pigment cells are found in the delicate connective tissue within the interstices of the cribriform modiolus in lower mammals (Wolff 1931) Neither has pigment been observed in the walls of the saccule of pigmented guinea pigs (La Ferriere et al. 1974) An abundance of pigment proportional to the pigmentation of the fur (Beck 1961) can be seen in most guinea pigs in low magnification in the area of the spiral ligament and the stria vascularis (Stahle Engström Högberg 1973) Conspicuous pigment cells also liberally adorn the walls of the utricle the ampullae and the semicircular canals (La Ferriere et al 1974) In rats a rich occurrence of pigmented cells in the stria vascularis has been observed by Hilding & Ginzburg (1977)

Kimura (1969) has described a close relationship between the dark cells and the subepithelial layers of melanocytes in the vestibular labyrinths of vertebrates such as the guinea pig bat opossum monkey and man The dark cells formed a distinct pattern in the ampullae utricle and common crus Melanocytes were organized subepithelially in patterns similar to those of the dark cells in all pigmented animals The cell processes and even the cell bodies of the melanocytes were occasionally found among the basal infoldings of the dark cells On the basis of their ultrastructural appearance the dark cells have been attributed a significant role in the active transport of electrolytes in the vestibular labyrinth (Kimura 1969) Similar qualities have been observed in the dark cells in the endolympha

tic sac (Lundquist, 1976 Harada & Gafar 1976) where subepithelial pigments have been revealed in experimental animals (Lundquist et al 1964) The resemblance in the distribution of pigment in the internal ear between different species of experimental animals and man makes us believe that general conclusions might be drawn from the experiments on young rats and/or from the experiments on human temporal bones and eyes

Preliminary experimental and therapeutic trials

The observation of apparent accumulation of chloroquine in the pigment of the labyrinth led us to think that this characteristic of melanin could be of value for therapeutic purposes Stimulated by earlier reports (Bárány 1935 Fowler 1953 Gejrot 1963) on the mitigating effect of intravenously administered local anaesthetics on tinnitus we assumed that this effect was due to some local action of the anaesthetics upon the internal ear We started with a short experimental as well as a clinical investigation of lidocaine which was accumulated in the modiolus in young pigmented rats Albino rats on the other hand showed no accumulation at all of labelled lidocaine in the internal ear (Englesson et al 1976)

These experiences led to some therapeutic trials in patients with severe and disabling tinnitus Relief was achieved for up to ? months after high-dose lidocaine treatment (Englesson et al 1976) The limited knowledge of acute and late side-effects of high-dose lidocaine therapy motivated the clinical study reported in the following

1 HIGH DOSE THERAPY WITH LIDOCAINE

A Clinical Toxicological Study in Man

Hearing and vision were studied in two different categories of subjects in order to analyse acute and late side-effects. (1) The first was a group of 9 patients who were given large doses of lidocaine as a general anaesthetic during middle ear reconstructive surgery namely 6 mg per kg body weight per hour intravenously The total dose of lidocaine ranged between 750 and ? 600 mg. Hearing and vision were tested in all cases shortly before surgery A second test was performed on the first day after surgery in 4 cases and within a week after surgery in the remaining 5 (?) The second group comprised 11 children treated with lidocaine for convulsions during the neonatal period (Norell & Gamstorp 1970) The dose given was initially 4 mg per kg body weight per hour intravenously with a gradual reduction over a period of 4–5 days The dosage varied between 148 and 696 mg per kg body weight The total amount of lidocaine administered to these children ranged between 394 and 2784 mg. The hearing and visual examinations which were intended as a study of the late effects were performed 5–7 years after the treatment

The test methods for the two groups were essentially the same Hearing was measured with a pure tone audiogram in all cases The patients in the first group (the acute study) also underwent speech audiometry

The ophthalmological examination comprised tests of visual acuity and colour vision examination of the optic media, and ophthalmoscopy The first group also underwent a visual field examination with red stimuli for central isopters using the Goldman perimeter

The results were as follows In the first group (study of acute effects) no differences were found in hearing and vision on comparing the immediately pre and postoperative results

In the follow-up of the 11 children exposed to large doses of lidocaine in the neonatal period the ophthalmological examination showed no signs of disturbances which could be related to the treatment Two of the children were mentally retarded and could not be completely examined Some of the children had defective visual acuity which could be related to other circumstances Hearing tests revealed a unilateral sensorineural hearing loss in one case All other children had normal hearing.

II LIDOCAINE BUPIVACAINE AND CHLORPROMAZINE AFFINITY TO MELANIN IN EXPERIMENTAL ANIMALS AND IN TISSUE SECTIONS

Material and Methods

Labelled compounds ^{35}S-chlorpromazine with a specific activity of 7 2 mCi/mmol was obtained from the Radiochemical Centre Amersham England It was dissolved in 28 mM HCl with a molar ratio of 1 1 thus yielding the hydrochloride

Bupivacaine hydrochloride (kindly supplied by the manufacturer AB Bofors Nobel Pharma Södertälje Sweden) was tritiated by catalytic hydrogen exchange in solution with tritium gas yielding a specific activity of 5 7 Ci/mmol (Radiochemical Centre Amersham England) The ^{3}H bupivacaine hydrochloride was stored at −20°C dissolved in ethanol until used The injectable solution was prepared by evaporation of the ethanol and the residue was redissolved in distilled water The radiochemical purity was analysed by thin layer chromatography on silica gel in a solvent system of methanol 25% ammonium hydroxide (100 15 v/v) whereby no impurity was found ^{14}C lidocaine hydrochloride (specific activity 2 85 mCi/mmol) was provided from New England Nuclear Boston Mass USA

Animals Young pigmented hooded rats (Lister) were used on day 11–18 post partum Albino rats (Sprague Dawley) were used on day 13–14 post partum

Whole-body autoradiography Whole-body autoradiography was performed as described by Ullberg (1954 1977) Pigmented and albino rats were injected intraperitoneally with 20 μCi of ^{35}S-chlorpromazine hydrochloride

The hooded rats were killed 20 min 1 hour 4 hours 1 day 4 days and 14 days and the albino rats 1 hour 1 day 4 days and 8 days after the injection by an overdose of chloroform

Subsequently all animals were mounted in a gel of carboxymethyl cellulose followed by rapid freezing in a mixture of solid carbon dioxide and hexane (−78°C) From every rat 20 μm and 60 μm thick whole-body sagittal sections were cut on tape (Minnesota Mining and Manufacturing Co No 688) After being freeze-dried at a temperature of −20°C, the sections were apposed to X ray film (Industrex C and Kodirex Kodak) for exposure. A series of young pigmented and albino rats, identical with the above were injected i.p. with 900 μCi per rat of ^{3}H bupivacaine hydrochloride The survival times were similar to those of the previous series 20 μm thick sections were cut and placed on ^{3}H film (Ceaverken Strängnäs Sweden) which is an X ray film lacking the antiscratch gelatin layer and therefore sensitive to tritium radiation in gross autoradiography (Larsson & Ullberg, 1977*a b*)

In vitro autoradiography To investigate the uptake of ^{35}S-chlorpromazine ^{3}H-bupivacaine and ^{14}C lidocaine in the melanin of the human eye and the internal ear *in vitro* tape mounted sections from these organs were incubated in aqueous solutions of the three compounds and then autoradiographed

All sections were allowed to freeze-dry The incubation solutions consisted of 1.5 μCi ^{35}S-chlorpromazine hydrochloride 250 μCi ^{3}H bupivacaine hydrochloride or 2 μCi ^{14}C lidocaine hydrochloride dissolved in 70 ml distilled water respectively The sections were submerged for 10 minutes in 20 ml portions of incubation solution at room temperature and rinsed for 60 minutes in running tap water and then air-dried Autoradiograms were made by apposition of the sections to appropriate X ray films (Industrex-C (Kodak) for ^{35}S and ^{14}C the ^{3}H film (Ceaverken) was used for ^{3}H) After exposure the sections were stained according to the Masson-Fontana method for melanin and subsequently mounted under cover glasses in Euparal®

In vitro investigation Melanin from beef eyes was prepared by a mechanical method described by Potts (1964) The reactions of the pigment with chlorpromazine hydrochloride and bupivacaine hydrochloride respectively

Table I *Semiquantitative evaluation of whole-body autoradiograms after intraperitoneal injections of ^{35}S-chlorpromazine ^{3}H-bupivacaine and ^{14}C-lidocaine respectively in young hooded rats*

The uptake in three different parts of the internal ear (modiolus, stria vascularis and vestibule) is related to the uptake in the eye which has been assigned the highest value throughout (+++). The scale used is relative in the increasing order − + ++ +++

Drug	Survival time	Site of accumulation: Modiolus	Stria vascularis	Vestibule	Eye
^{35}S-chlorpromazine	20 min	++	+	+	+++
^{35}S-chlorpromazine	1 hour	+	+	+	+++
^{35}S-chlorpromazine	4 hours	+	+	+	+++
^{35}S-chlorpromazine	1 day	+	++	+	+++
^{35}S-chlorpromazine	4 days	+	++	+	+++
^{35}S-chlorpromazine	14 days	+	+	−	+++
^{3}H-bupivacaine	20 min	+	+	+	+++
^{3}H-bupivacaine	1 hour	+	+	+	+++
^{3}H-bupivacaine	4 hours	+	+	+	+++
^{3}H-bupivacaine	1 day	+	+	+	+++
^{3}H-bupivacaine	4 days	+	+	+	+++
^{3}H-bupivacaine	14 days	+	+	+	+++
^{14}C-lidocaine*	1 hour	+	−	−	+++
^{14}C-lidocaine*	4 hours	+	−	−	+++
^{14}C-lidocaine*	1 day	+	−	−	+++
^{14}C-lidocaine*	4 days	−	−	−	+++
^{14}C-lidocaine*	14 days	−	−	−	+++

* Report on lidocaine has been published earlier (Englesson et al. 1976)

Note there is no quantitative correspondence between different survival times or different drugs concerning the accumulation in the eye

were produced mainly according to the method of Potts (1964) 2.5 μmoles of the substance dissolved in 5 ml distilled water 1 ml M/15 Sorensen buffer (pH 7 0) and 1 ml of the 10 mg/ml-pigment suspension were mixed and incubated for 45 minutes at room temperature In that time an equilibrium was established between the melanin-bound and non-bound substances and the two fractions were separated by centrifugation at 35 000 *g* for 10 minutes in a MSE 25 high-speed centrifuge The concentration of the substance in the supernatant was measured by a Hitachi Perkin-Elmer 124 spectrophotometer at 307 nm for the chlorpromazine and at 235 nm for the bupivacaine By referring these concentrations to corresponding values for the initial concentrations (obtained by replacing 1 ml pigment suspension with 1 ml distilled water in the reaction mixture) the melanin affinities could be calculated The experiments were carried out in duplicate

Results

Pigmented rats

Accumulation of labelled lidocaine bupivacaine and chlorpromazine was studied at four points—the modiolus stria vascularis vestibule and eye

The total radioactivity after survival times of 20 minutes to 14 days was evaluated semiquantitatively (Table I) A very strong uptake in the eyes dominated all autoradiograms (Fig. 1) Distinct accumulation was noted in the *modiolus for all three substances* (Figs 2 3 4) Local as well as specific variations in the labyrinthine uptake of the three compounds were observed. Chlorpromazine showed the most pronounced accumulation to the stria vascularis in comparison with the two other compounds (Fig. 4) This compound had the

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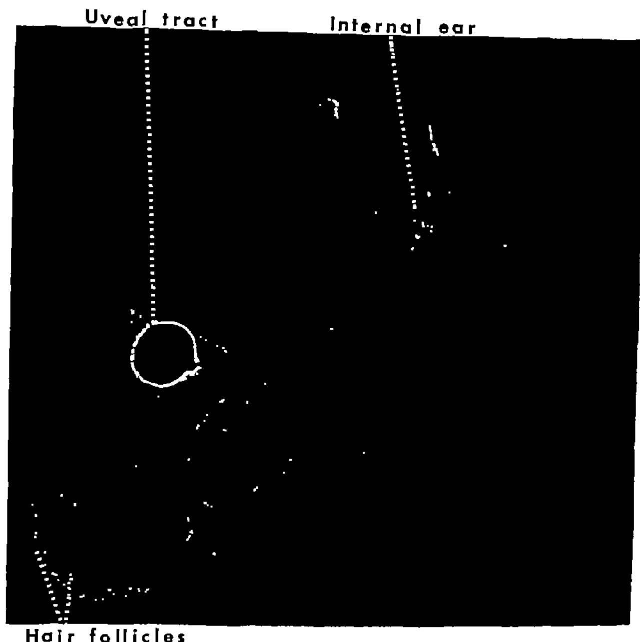


Fig 1 Detail of an autoradiogram of a young hooded rat 4 hours after an intraperitoneal injection of H bupivacaine There is a high accumulation in the uveal tract the internal ear (stria vascularis and modiolus) and the hair follicles ×5

strongest affinity to melanin and lidocaine the weakest Chlorpromazine and bupivacaine were discovered in all three studied parts of the internal ear while lidocaine was found in the modiolus but not in the stria vascularis or the vestibule As regards regional differences a tendency to a stronger accumulation in the cochlea than in the vestibule was noted (Figs 2 and 3)

The duration of the retention of the three compounds in the internal ear varied Chlorpromazine and bupivacaine were both observed from 20 minutes after injection up to 14 days Lidocaine on the other hand was not identified at its single place of uptake in the modiolus 4 days after injection (Table I)

Albino rats

No accumulation or retention was observed in the eyes of albino rats except for very weak radioactivity at one day in the rat injected with ^{35}S-chlorpromazine



Fig. 2. Detail of an autoradiogram showing the internal ear young hooded rat 4 days after an intraperitoneal injection of ^{3}H-bupivacaine. Note the high accumulation in the stria vascularis (*white arrows*) and modiolus. ×6



Fig. 3. Detail of an autoradiogram of young hooded rat 1 hour after an intraperitoneal injection of ^{35}S-chlorpromazine. There is high accumulation in the internal ear. Besides the apparent uptake in the modiolus, the spiral osseous laminae are delineated as well as the stria vascularis (*white arrows*), at several levels. × 1

After the injection of ^{35}S-chlorpromazine there was a slight radioactivity at 1 hour and 1 day in the modiolus and stria vascularis of the albino rats but at 4 days the whole internal ear was empty. The accumulation of ^{3}H-bupivacaine in the internal ear was very slight and was limited to the stria vascularis at 1 hour and 1 day. No radioactivity was observed in the modiolus or vestibule and at 4 days the whole internal ear was emptied. There was no uptake in the internal ear of albino rats injected with ^{14}C-lidocaine as has been reported by our group previously (Englesson et al. 1976).

In vitro investigations

These studies were carried out in two ways, (1) using isolated bovine eye melanin and (2) by incubation of human temporal bone and human eye sections.

(1) Chlorpromazine showed a high adsorption to isolated bovine eye melanin whereas the adsorption of lidocaine and bupivacaine

Table II. *Adsorption of different compounds to isolated bovine eye melanin*

Compound	Relative adsorption in per cent	Compound (Lindquist 1973)	Relative adsorption in per cent
Lidocaine	12	Salicylic acid	0
Bupivacaine	19	Streptomycin	60
Chlorpromazine	86	Quinine	68
		Kanamycin	89
		Chloroquine	97

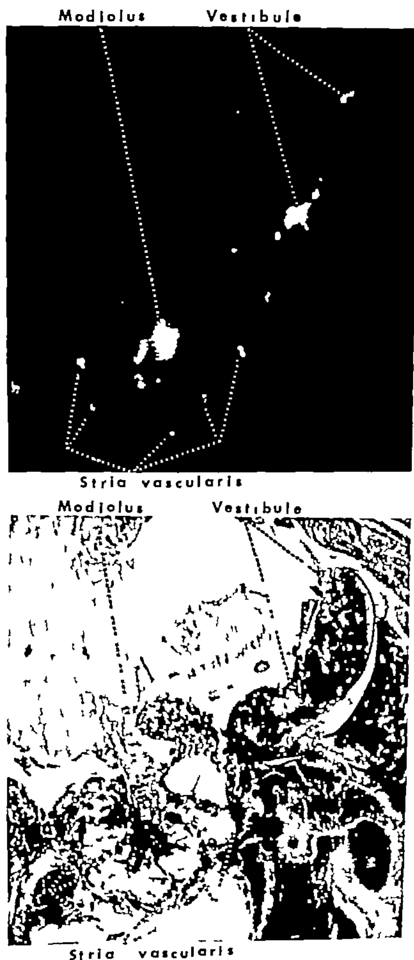


Fig. 4. Detail of an autoradiogram (upper figure) with the corresponding section (lower figure) of a young hooded rat 4 days after an intraperitoneal injection of ^{35}S-chlorpromazine. Note the high accumulation in the melanin of the internal ear. The extent of the cochlea is apparent from the regular arrangement of the uptake in the stria vascularis. The localized radioactivity outside the cochlea correspond to vestibular structures and

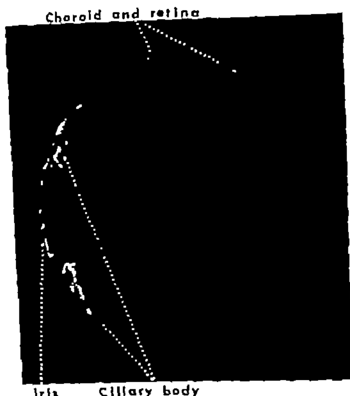


Fig 5 Autoradiogram showing the affinity of ^{35}S-chlorpromazine for human eye melanin *in vivo*. The eye is sagittally sectioned. The apparent uptake of radioactive substances in the iris and the ciliary body corresponds to the high melanin content of these tissues. 4

to this melanin was much lower (Table II) A comparison with other previously studied compounds such as antibiotics of the aminoglycoside type and chloroquine is presented in the table

(2) The autoradiograms of human temporal bone sections from a newborn infant and a 76-year-old man showed no definite accumulation of labelled substances in the internal ear These results are not quite conclusive owing to technical difficulties in preparing qualitatively acceptable whole temporal bone sections Experiments with this purpose are in progress

The eye from a 68-year-old man showed an apparent accumulation of chlorpromazine in the uveal tract (Fig. 5) and a scanty accumulation of bupivacaine and lidocaine at this site

DISCUSSION

Melanin is present in the internal ear in both pigmented animals and in man and the amount of melanin in the internal ear is related to the amount of pigment in the skin and iris (Bonaccorsi 1965 Anticaglia 1970) The melanin in the internal ear can occur both as pigment in the melanocytes (mainly in the cochlea) and as free granulations and clusters 7–10 μm long (mainly in the vestibular portion)

Several reports imply that hearing is better preserved in coloured than in white populations Bunch & Raiford (1931) studied the hearing in negro and in white American hospital patients with the same cultural background The hearing was found to be better in the black than in the white patients in the frequency range above 2000 Hz and the differences increased with age Post (1964) evaluated hearing examinations of American conscripts for the Second World War and noted that coloured males above middle-age had better hearing for high-frequency tones than white males of the same age groups Roberts & Bayliss (1967) studied a material from the Health Examina-

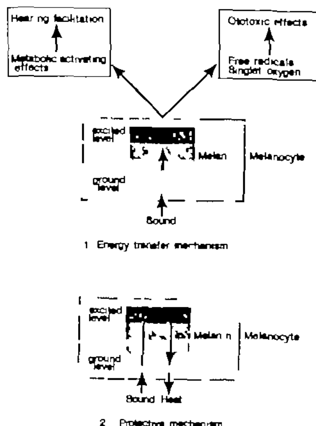


Fig 6 Melanin can be regarded as an amorphous semiconductor with a high redox potential With these properties it is theoretically conceivable that melanin may take part in different processes in the internal ear as exemplified in this illustration

tion Survey by the National Center for Health Statistics In summary they found better hearing in coloured groups especially men over 30 years of age In 1972 Karsai investigated 836 dock workers with a relatively uniform background He noted significantly better hearing for treble tones in the coloured men than in the white ones Shephard & Goldstein (1964) made a study of coloured and white individuals both men and women and reported in contrast to all the authors cited above that the white groups had better hearing Each of their groups only comprised about 20 persons however and the reliability of the results may therefore be questioned

The transient effect of exposure to noise so called temporary threshold shift (TTS) was examined by Tota & Bocci (1967) Pure tone stimulation with 100 dB/1 000 Hz for 3 minutes was used and a significantly lower TTS was found in brown-eyed than in blue-eyed individuals They considered the possibility that the melanin in the stria vascularis might have a protective effect whereby brown-eyed persons would be more resistant to noise-damage than blue-eyed persons Hood et al (1976) have since clearly confirmed the finding that the melanin content of the iris as determined by eye coloration bears a direct relationship to the temporary threshold shift The same authors also found that for low levels of stimulus intensities there was little difference between brown-eyed and blue-eyed persons but with higher intensities the differences increased being highly significant at the 120 dB level From their results they concluded that at lower stimulation levels (up to 100 dB) the function of melanin would be in auditory adaptation and at higher levels (120 dB) it would be in auditory fatigue They therefore postulated a possible protective effect of melanin for high-noise levels

Pigment defects with a genetic background are often found to be correlated to hearing loss the best known example being the Waardenburg Klein syndrome The hearing loss in these general pigment defects can vary from moderate sensorineural impairment to complete deafness In these conditions pathological changes of the Scheibe" type are found in the cochlea and saccule of the internal ear besides a lack of pigment there is an incompletely differentiated scala media in the cochlea and a poorly differentiated sensory epithelium in the saccule (for review see Deol 1968)

With knowledge of the physico-chemical properties of melanin the idea that it may be involved in the hearing process has been put forward (Proctor et al 1974) As seen from the above review of the literature findings have been made that may imply that hyperpigmentation has a positive impact and pigment deficiency a negative impact on hearing. In the light of these observations we consider that the possible role of melanin in the hearing and balance functions should be discussed and analysed further

Melanin is a polymer of ring structures containing indole 5 6-quinone with tyrosine as its precursor In the pigment granules the melanin is bound to proteins Melanin can be regarded as an amorphous semiconductor and has a high redox potential The electronic properties of the melanins are probably best explained in terms of the amorphous semiconductor theory (McGinness & Proctor 1973 McGinness et al 1974) in which electronic states are closely coupled to vibrational modes of the polymer Theoretically with these properties melanin might participate in different processes in the internal ear (Proctor et al 1974) Thus (1) it could act as an energy transformer e g. transforming mechanical to electrical energy and (2) it could have a protective effect in that the sound energy peaks could be cut off through excitation of the melanin with subsequent reversion to the resting state during emission of thermal energy (Fig. 6)

The energy transfer properties of melanin are influenced by coupling to chlorpromazine (Corry et al 1976) For example the change of threshold-switching potentials (the potential at which the conductivity of melanin is abruptly changed) is decreased by a low concentration of chlorpromazine and increased by a high concentration of the same compound

By ultrasonic treatment of melanin-containing melanoma cells in cell cultures another effect of chlorpromazine affinity to melanin has been tested (McGinness et al 1976) These authors have shown that the toxic effect of ultrasonic irradiation is potentiated if chlorpromazine is bound to the melanin

Theoretically the two postulated properties of melanin energy transfer and sound protection may be altered by substances with melanin affinity Both a beneficial and a noxious effect of substances with melanin affinity therefore have to be considered

Treatment of severe tinnitus by intravenously administered lidocaine has recently been reported (Englesson et al 1976 Melding et al 1978) We have now extended these experiments by studying in detail the accumulation of lidocaine chlorpromazine and bupivacaine in animal and human melanin The purpose was to thoroughly evaluate the theoretical possibility of treating severe tinnitus with drugs that bind to melanin The three tested substances all became adsorbed to inner ear melanin with chlorpromazine showing the highest uptake Theoretically they would therefore seem suitable for trial in the treatment of various internal ear disorders

It is also noteworthy that some antihistamines are effective motion sickness suppressors for example the phenothiazine derivatives promethazine and its quaternary analogue *N*-hydroxyethylpromethazine These preparations have a well known anticholinergic and sedative effect No specific effect on the internal ear has been discussed previously however N-hydroxyethylpromethazine binds to the melanin in the internal ear of young rats to a great extent (Larsson 1978 personal communication) It cannot be excluded that there may be a connection between the motion sickness suppression ability of these drugs and their property of adsorption to melanin in the vestibular part of the internal ear

The possible ototoxic effect of these substances finally must be taken into consideration Clinical studies of lidocaine however revealed no acute or late effects on the internal ear or the eye

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die Verteilung und das Verhältnis von markiertem Lidocain, Bupivacain und Chlorpromazin zum Melanin im Innenohr nach intravenöser und intraperitonealer Injektion wurden mit "whole-body"-Autoradiographie untersucht Sowohl junge Ratten mit pigmentiertem Pelz als auch Albinoratten wurden studiert. Die pigmentierten Ratten zeigten die stärkste Anhäufung von Chlorpromazin, mehr im cochlearen als im vestibularen Teil Die anderen beiden Substanzen waren gleichmäßig im inneren Ohr verteilt Nach nur einer Injektion von Chlorpromazin und Bupivacain waren diese Substanzen nach 14 Tagen welches die längste Überlebenszeit war noch an das Mela-

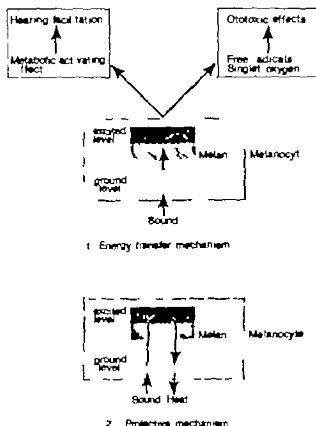


Fig. 6. Melanin can be regarded as an amorphous semiconductor with a high redox potential. With these properties it is theoretically conceivable that melanin may take part in different processes in the internal ear, as exemplified in this illustration.

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nin im Innerohr gebunden Degegen war Lidocain schon nach 4 Tagen nicht mehr nachweisbar Eine starke Aufnahme und Retention der drei Substanzen wurde in den Augen der pigmentierten Tiere beobachtet. Bei den Albinos fand man eine sehr schwach vorübergehende Aufnahme von Chlorpromazin und Bupivacain im Innerohr jedoch nicht von Lidocain Bei Beobachtungen *in vitro* von isoliertem Rinderaugen-Melanin fand man eine beträchtlich höhere Adsorption von Chlorpromazin als von Lidocain und Bupivacain Eine schwache Aufnahme wurde bei Versuchen *in vitro* mit Menschenaugen gefunden Klinische Teste nach großen Dosen Lidocain zeigten keine akuten oder späten Hör oder Sehschaden Die Teilnahme des Melanins an verschiedenen basalen Labyrinthfunktionen wie des Energie Übertragungs-Mechanismus und des Laut Schutz Mechanismus wird an Hand der erhaltenen Resultate diskutiert Weiterhin ist die Theorie aufgestellt worden daß die Melaninaffinität gewisser Substanzen sowohl von therapeutischer als auch ototoxischer Bedeutung sein kann

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'able I *Operathe procedures age and pre-perative hearing levels*

peration	Age (years) Mean	Range	Average pure tone bearing level for 0.5 1 and kHz Mean	Range
4iddle fossa estibular neurectomy N=23	44	.3–61	58	13–75
Translabyrinthine eighth nerv section or labyrinth destruction $N=16$	47	28–63	76	45–105
Saccus decompression, N=4	46	29–60	39	23–58

onged periods of time This has clearly been a restraining factor in our policy with regard to surgery for Meniere s disease which we have imited to those cases in which the vertiginous symptoms have become unbearable (Palva et al 1976) Our observations led to the conclusion that surgical modalities other than labyrinth destruction or vestibular neurectomy have little lasting effect on the patient s most disturbing symptom vertigo

Continuing along this line we have limited our surgery to advanced stages of the disease and on the basis of earlier experience adopted vestibular neurectomy as the principal method of surgical treatment In addition endolymphatic sac surgery has been carried out in bilateral cases and in cases where the sole hearing ear was affected by Meniere s disease The patients operated upon have been followed regularly and their subjective symptoms and objective data recorded

MATERIAL AND METHODS

The material consists of 42 patients (43 operated ears) with Meniere s disease The age range is shown in Table I the majority of the patients were between 40 and 50 years old Nineteen were female and 23 male patients The disease was bilateral in 4 patients In the neurectomy groups the few cases with good hearing had a long history of incapacitating vertigo

All patients were studied by pure tone and speech audiometry and most patients by means of Fowler s alternative loudness balance recruitment test stapedius reflex and adaptation tests and vestibular function tests The data on preoperative average (500 1000 2000 Hz) pure tone thresholds are also given in Table I All patients had a depressed vestibular function in caloric tests The glycerol test (Klockhoff & Lindblom 1966) has been used only since 1977 and none of the cases operated on showed any significant improvement after intake of glycerol Surgery was done between 1974 and 1977 and all cases have had at least one year of follow-up observation

In the middle fossa approach the opening to the cerebrospinal fluid space was occluded

Table II

No of surgical interventions	Neurectomy 23	Destruction + neurectomy 16	Saccus 4
Hearing			
improved	3		
no change	15		1
worse	1		1
complete loss	3	16	1
no data	1		1
Vestibular function			
extinguished	23	16	
impaired			1
no change			2
no data			1
Vertigo			
no symptoms	18	10	1
milder symptoms	5	4	3
no change		1	
more severe		1	
Preoperative working			
full ability			
partial disability	19	9	3
total inability	4	7	1
Postoperative working			
full ability	19	13	
partial ability	1	1	
total inability	3	2	2

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VESTIBULAR NEURECTOMY AND SACCUS DECOMPRESSION SURGERY IN MENIERE'S DISEASE

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Abstract. Results of vestibular neurectomy, total eighth nerve section and saccus decompression in 42 patients with Meniere's disease are reported. Vestibular nerve section was found in isolated cases to be a very effective method of abolishing the symptom of vertigo. Hearing is not affected but may be lost owing to opening of the vertical canal or disruption of blood supply. Saccus surgery might be the surgical treatment of choice in early cases with good hearing, but in patients with fixed non-fluctuating hearing loss, rehabilitation can be effected only by vestibular neurectomy. In bilateral cases either sac surgery or the use of vestibulotoxic drugs is advised.

As regards its etiology, Meniere's disease has always been an unsolved mystery. The gross histopathological finding, dilatation of the membranous endolymphatic system (Hallpike & Cairns, 1938), has given rise to much speculation, particularly concerning the choice between conservative and surgical treatment. Torok (1977) recently reviewed 823 articles on Meniere's disease published in the years 1957–75 and gave a rather gloomy picture of its management. All conservative forms of treatment had one aspect in common: they claimed to affect a cure of the vertiginous attacks in 60 to 80% of patients, at least for a limited observation time lasting from a few months up to a few years. Similarly, all surgical modalities discussed were reported to be successful to about the same extent for similar follow up periods. Schuknecht (1977) even suggested that it is the surgical tissue insult to the labyrinth which together with the concomitant inflammatory and biochemical changes causes an alteration in the function of cells controlling fluid physiology. Thus, the symptoms of Meniere's disease could be alleviated, at least temporarily.

Although there are no data from man that endolymphatic sac obliteration has any effect upon the development of endolymphatic hydrops, data from guinea pigs (Kimura, 1967) and cats (Schuknecht et al., 1968) have shown hydrops to occur. This is indirectly supported by records of surgery on human ears, many of which testify to the difficulties encountered in establishing a lumen in the sac (Shambaugh et al., 1969). In cases of acoustic neuroma, on the other hand, a lumen is invariably found (Palva, 1978). Obviously some poorly known events occurring in the endolymphatic sac during the course of Meniere's disease cause the gradual disappearance of the sac epithelium. A corollary of this reasoning is that sac surgery, when attempted, should be done early, i.e. after the first attacks, before permanent loss of the resorptive function of the sac can establish itself.

The problem with early surgical treatment for Meniere's disease is the circumstance that a large proportion of the patients to be operated on manage fairly well with or without various conservative methods of management. One cannot be really sure subsequently whether early surgery has actually contributed to the cure at all, or whether it was the natural benign course of the disease or medication that caused the symptoms to disappear for pro-

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estibular function remained unchanged The lassification of the material as suggested in tes above-mentioned recommendation is resented in Table III

The tables indicate that results of middle ossa neurectomy were 100% successful in lleviating or controlling the vertigo Laby inth destruction and neurectomy resulted in elief of vertigo in 90% of the patients while in (10%) vertigo either remained the same or was made worse This was due to poor selec ion of cases. In both patients the disease ~ing bilateral was activated in the better non- perated ear

A brisk spontaneous nystagmus towards the healthy ear was observed in all neurectomy cases during the first postoperative days It ibated gradually but could be seen regularly during the first postoperative months in ENG-recordings. In the one year follow-up ex aminations only about 20% of the patients showed a weak spontaneous nystagmus less than 4 /s towards the nonoperated ear In comparison with the preoperative values the postoperative reactions to both cold and hot water were similar in 2/3 of the cases in the healthy ear and in 1/3 of the cases the healthy ear showed differences compatible with the directional preponderance observed preopera-tively

COMMENT

Although vestibular neurectomy has occa sionally been employed in bilateral cases (Fisch 1976) we think that this is inadvisable Abolishing the function of one labyrinth leav ing the compensatory process to depend on the other though not equally affected laby rinth seems to involve too difficult an adjust ment for these incapacitated persons In bi-lateral cases therefore we now do bilateral saccus decompression and if relief is not there by obtained we abolish most of the function with ototoxic drugs It would of course be possible to perform bilateral nerve section but even when the possible compromise of the cochlear function is disregarded patients with Meniere s disease might find it very difficult to manage as their balance would depend on vision and deep sensitivity alone

In unilateral cases compensation seems to occur quite rapidly after vestibular neurec tomy for the loss of one balance organ but it is of paramount importance to stress the neces-sity of early active exercises These can be started very early The patients are en-couraged to walk—aided if necessary—on the second or at the latest on the third postopera-tive day Allowing the patients to lie in bed for several days greatly delays postoperative re-habilitation Another important point before the operation is undertaken is to make the pa tient understand that his active effort is needed for recovery and the surgeon must be con vinced on discussion with the patient that the latter is really willing to resume his earlier activity The senior author has refused to do any surgery on a few occasions when it be came obvious that the patient had no genuine desire to start working but viewed the "unsuc cessful" operation as a further final proof that he was totally incapable of working and ex pected to receive a lifelong pension even at a relatively young age

Both vestibular neurectomy and labyrinth destruction and 8th nerve neurectomy were successful in abolishing the vertiginous attacks in all unilateral cases Several patients re ported some occasional unsteadiness and slight, slow up and down motion but these symptoms were felt to be of minor importance These results are essentially similar to those reported by Fisch (1976) for example

As to hearing the basic process is not af fected by this surgery and major improve ments cannot be expected. However some patients suffered total loss of hearing which either was due to inadvertent opening of the semicircular canal or resulted from some cause probably related to changes in the microcirculation in the nerve The effect of total eighth nerve section on tinnitus is uncer tain and it is our impression that there will be

Table III *Results of one year follow up classified as recommended by the Committee on Hearing and Equilibrium*

Operation	Postoperative group re hearing and vertigo				
	A	B	C	D	Total
Neurectomy	4	15	4	0	23
Destruction and neurotectomy	0	0	14	2	16
Saccus decompression	0			0	4
Total	4	17	20		43

with a piece of temporal muscle in the translabyrinthine approach with abdominal fat

For decompression of the endolymphatic sac the steps after the simple mastoidectomy has been performed are exposure of the cerebellar dura medial to the descending part of the sigmoid sinus and removal of the bone over the sac Primary identification of the posterior semicircular canal is not attempted as it is considered to involve substantial hazards in surgery There is really no difficulty in establishing the area of the endolymphatic sac but it is a matter of considerable doubt whether its real lumen can be found The sac is opened up with a sickle knife starting from its posterior margin close to the sigmoid sinus If there is a good lumen opening of it discloses a fairly large free space under the operating microscope During the early years of decompression surgery (Palva et al 1976) a small silicone tube was inserted into the sac lumen but this has now been replaced by one or two silver clips which are fixed to the upper lid of the sac to guarantee a permanent fistula into the mastoid Opening of the sac into the CSF space is not considered advisable

RESULTS

In the pre operative testing the majority of the patients presented findings generally considered typical of cochlear hearing-loss However there were also tests which yielded information contradictory of a cochlear lesion In Fowler's loudness balance test 22 ears showed recruitment of loudness while 2 showed no recruitment Adaptation of threshold was normal (30 dB or less) in 21 ears but pathologic (over 30 dB) in 6 ears In the reverse frequency Békésy audiogram 22 ears showed normal overlapping while in 4 ears the curves were separated as is usually the case in retrocochlear pathology The stapedius reflex threshold was normal (ad 100 dB HL) in 17 cases elevated (up to 120 dB HL) in 3 ears and unmeasurable (over 120 dB HL) in one.

Vestibular and total eighth nerve neurectomies formed the two main groups of surgery and only a small number of saccus operations were performed (Table II) In addition there was one case in which vestibular neurectomy had been planned but in which a large vein was found high up in the meatus and ligated with disappearance of symptoms The group of eight nerve section includes two cases in which only total labyrinth destruction down to the meatus was made without cutting of the nerves

The postoperative course in the nerve section material was uncomplicated in all cases, the early symptoms of severe vertigo abating in 1 to 3 days The patients all had a recovery period of 2 months before they were advised to start working again Facial paralysis developed during the first postoperative week in 6 patients but all recovered well

The results of the one year follow up are given in Tables II and III Changes in hearing and vertigo are given according to the recommendations of the Committee on Hearing and Equilibrium (1972) It appears that hearing was as a rule unaffected in the middle fossa vestibular nerve section group excluding 3 patients who lost their remaining hearing In 2 of these ears the cause was accidental opening of the vertical canal and in one the cause was unknown (the preoperative hearing levels in these cases were 78 90 and 82 dB) Vestibular function was totally extinguished in all ears In the ear in which the large vein was ligated

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CERVICAL AND VESTIBULAR AFFERENT CONTROL OF OCULOMOTOR RESPONSE IN MAN

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Abstract Oculomotor response in the absence of vision has been compared in group of 12 normal humans in two experimental conditions testing ( ) the vestibulo-ocular reflex by whole-body oscillation on turntable and (*b*) the cervico-ocular reflex by oscillation of the body with the head held stationary The stimulus was sinusoidal oscillation (peak angular velocity ± 50°/sec) at frequencies between 0_ and 1 3 Hz The slow-phase eye movements of the vestibulo-ocular response were compensatory for head movement and showed mean gain of 0 54–0 90 increasing with frequency The cervico-ocular response was found to be very variable The slow phase eye movements were of low velocity (mean gain 0 05) and did not generally compensate for body movement During neck torsion, some subjects exhibited large overall eye deviations composed of both slow and fast phase eye movements

In 1906 Barany first made observations which implied the existence of spinal afferents to the oculomotor system by eliciting a coordinated deviation of the eyes towards the direction of trunk movements when the head was held stationary in space Similar responses were reported by de Kleijn (1921) and Grahe (1926) The existence of neural pathways projecting from the cervical receptors to the vestibular nuclei was demonstrated by Brodal et al (196_) and more recently Rubin et al (1975) have recorded from units in the vestibular nuclei of the cat which responded to both vestibular and neck torsional stimuli. Hikosaka & Maeda (1973) were also able to record, in the abducens nerve of the cat activity resulting from electrical stimulation of the contralateral vestibular nerve which was inhibited by stimulation of the contralateral neck joint It seems probable that it is the neck joint receptors rather than muscle spindles which are the primary source of neck afferents involved in the cervico-ocular response (McCouch et al 1951 Biemond & de Jong 1969)

In a recent review Dichgans (1974) concluded that neck to-eye afferents almost certainly cooperate with the vestibulo-ocular reflex to stabilise the eye on visual targets during both active and passive movements of the head on the trunk However it is important to distinguish between phasic and tonic reflexes in this respect. In newborn children torsion of the neck causes horizontal ocular movements (Barany 1918) and as long as the torsion is maintained the eyes remain in a compensatory position But in adults when optic fixation is abolished the sustained tonic compensatory response is rarely observed (Grahe 1926)

More recently the nature of the phasic neck-eye reflex in humans has been studied by Meiry (1966) and Takemori & Suzuki (1971) Meiry concluded that during passive rotation of the trunk with the head fixed a slow-phase eye movement was generated which was essentially compensatory in nature and which had a gain of approximately 0 3 at frequencies below 0 1 Hz, decreasing to less than 0 1 at frequencies above 0 4 Hz. Meiry compared responses to both active and passive head movements and suggested that the vestibulo-ocular and cervico-ocular reflex responses were algebraically summed during voluntary head movements

no change in tinnitus in longstanding cases However there are certain patients with shorter tinnitus duration who experience great relief tinnitus either diminishing or changing in quality and therefore we plan to continue to do eighth nerve section in cases of very poor hearing In many instances the source of tinnitus is obviously at the level of the cochlear nuclei and thus lies outside the possibility of cure by surgery

The most encouraging aspect of treatment by vestibular nerve section is that the majority of these patients are able to resume their earlier work However as stress and emotional factors which are very much affected by physical fatigue definitely play a role we recommend the employer not to place these persons in shift work and not to employ them in work where noise levels are very high or strongly vibrating tools are used

Finally surgical treatment of Meniere patients might logically include as recently also suggested by Morrison (1976) more early saccus decompression operations in patients whose hearing is still normal or nearly normal during the periods between attacks Late surgery of the sac after near total occlusion of the lumen and fibrosis does not in our limited experience give expected relief From a physiological viewpoint decompression and opening of the sac attack the likely site of the disease *while neurectomy will remain* symptomatic treatment

ZUSAMMENFASSUNG

Neurektomie des Vestibularnerves totale Unterbrechung des 8 Hirnnerves und Dekompression des Saccus sind Methoden der operativen Behandlung in der Meniereschen Erkrankung an 42 Patienten die in diesem Rapport berichtet sind Seh gute Ergebnisse in der Behandlung des Schwindels waren mit Vestibularneurektomie zu gew n- nen Dabei war gewohnlich keine Anderung des Gehörs nachweisbar ausgenommen diejenigen Fälle wo n eine Offnung der vertikalen Bogengänge oder Du blutungsstörung verursachte Saccusoperation Ware Methode der Wahl in den fruheren Fällen sein, der eine gute Hörleistung haben aber Patienten, die e *dauernde nichtfluktuierende Schwerhörigkeit haben*, k man nur mit Hilfe von Vestibularneurektomie eine er nisvolle Behandlung anbieten Bei den bilateralen Fä werden entweder eine Saccusoperation oder vestib toxische Medikamente empfohlen.

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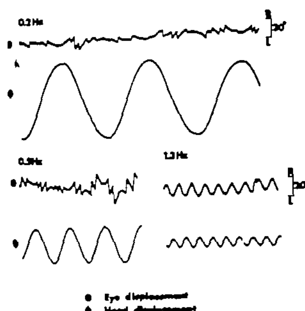


Fig 1 Typical responses of the vestibulo-ocular reflex to whole-body angular oscillation about the yaw axis at various frequencies of stimulation.

be carried out without any alteration in dark adaptation Such calibrations were performed at the beginning and end of each experimental condition During the experiment which was conducted in the dark, the subjects were instructed to stare blankly to the front with the eyes open.

The subjects were six male and six female volunteers all had normal mobility of the head and neck, and none had any known neuro-otological defect

Analysis

For the analysis of the results the analogue eye and turntable displacement signals from the tape recorder were first differentiated to obtain velocity and digitised for subsequent computer processing. Gain and phase relationships were obtained by directly comparing the head velocity with slow-phase eye velocity using a least square error technique All saccadic components and their associated points on the head velocity waveform were rejected from the regression analysis by a threshold exclusion process, so that no interpolation between successive slow-phase components was required Directional preponderance was also computed A particular advantage of this analysis procedure is that it is not dependent on a high fidelity stimulus waveform A minimum of 8 cycles of the stimulus waveform were analysed for each frequency and no part of any record was rejected.

RESULTS

Qualitative features of the vestibulo-ocular reflex

The eye movements resulting from whole body oscillation stimulating principally the horizontal semicircular canals are shown in Fig 1 The responses are those of one subject at three of the six stimulus frequencies but they are typical of the responses of all subjects

As noted previously (Merry 1966 Benson 1974 Hixson, 1974) the slow phase eye movements were essentially compensatory for head movement, the velocity being modulated in the same approximately sinusoidal manner as the input waveform The fast phase eye movements on the other hand were anticompensatory and in general biased the eye towards the direction of head movement, a feature

The vestibulo-ocular reflex plays an important part in eye stabilization during voluntary head movement in normal subjects Patients with loss of vestibular function exhibit impaired ocular stabilization (Bender & Feldman 1967) but there can be considerable adaptation and recovery of head-eye coordination (Atkin & Bender 1968) Dichgans et al (1973) showed that in the monkey the recovery of eye stabilization could be partly attributed to the cervico-ocular reflex which became potentiated following labyrinthectomy However it was noted that the gain of the reflex was less during passive than during active neck torsion

This paper describes experiments which were designed to test the vestibulo-ocular and cervico-ocular reflex responses of normal subjects over a range of frequencies (0 2–1.3 Hz) representative of normal head movement The analysis of the oculomotor response was carried out with the aid of a newly-developed technique and the results form a basis upon which to assess the effects of labyrinthectomy in man (Barnes 1978)

METHODS

Apparatus

The apparatus consisted of a rigid portable aluminium framework erected around a small low friction turntable on which a rigid padded seat and footplate were mounted The subject was secured into the seat with a full shoulder and lap harness

An adjustable aluminium helmet was secured on the head and two aluminium sections attached to the rear of the helmet enabled the head to be immobilised in either of two positions First when testing the response of the vestibulo-ocular reflex alone the helmet was clamped to the seat so that both head and body were coupled to the turntable Second the helmet was clamped to the rigid framework which enabled the body to be oscillated whilst the head was maintained in a fixed position thus stimulating the cervico-ocular reflex In this second configuration further stabilisation of the head was provided by the use of a pre-mou[illegible] dental bite which was rigidly attached to [illegible] adjustable crossbar coupled to the fram[illegible] work

Procedure

In both experimental conditions the mot[illegible] stimulus was a sinusoidal oscillation about th[illegible] yaw axis each subject being exposed to s[illegible] frequencies of oscillation between 0 ? and [illegible] Hz presented according to a balanced ra[illegible] domised design Turntable motion was pr[illegible] vided manually by the experimenter wh[illegible] stood behind the subject and rotated the cha[illegible] smoothly in an oscillatory manner The e[illegible] perimenter gauged the angular displaceme[illegible] of the turntable by viewing markings on th[illegible] turntable deck which were illuminated by [illegible] low intensity light source invisible to th[illegible] subject The requisite frequency of turntab[illegible] oscillation was obtained by synchronisatio[illegible] with a low intensity repetitive audio sourc[illegible] the peak amplitudes of angular displacemen[illegible] were chosen so that peak angular veloci[illegible] was maintained at approximately ±50°/s[illegible] throughout the frequency range The turn[illegible] table motion was lightly sprung enabling [illegible] smooth approximately sinusoidal wavefor[illegible] to be produced even at the lowest frequenc[illegible] (0 2 Hz)

The angular displacement of the turntabl[illegible] was transduced by a potentiometer and angu[illegible] lar acceleration of the subject s head wa[illegible] measured with an angular acceleromete[illegible] attached to the top of the helmet Eye di[illegible] placement was recorded by the electro-ocul[illegible] graphic technique using silver/silver chlorid[illegible] disc electrodes placed beside the externa[illegible] canthus of each eye All signals were recorde[illegible] on an FM tape recorder for subsequen[illegible] analysis

The subject wore red filter goggles through out the experiment This enabled calibration of eye movements to target light sources to

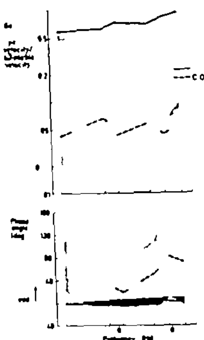


Fig. 3 Gain and phase relationships between slow phase velocity and turntable velocity for the responses of the vestibulo-ocular and cervico-ocular reflex to angular oscillation about the yaw axis

the predominant activity was contained within the saccadic components The slow-phase component was very small and in contrast to the response of the vestibular reflex the saccades formed the compensatory response in a very regular pattern In the results shown in Fig. 2, which are from one subject there is a preponderance of saccadic activity towards the left, although this was not explicable in terms of any spontaneous nystagmus. However this subject did report a sensation of increased stiffness of the neck when moved to the left.

The type III response which occurred in of the subjects (Fig. III) was typified by large amplitude pendular eye movements often asymmetric in form with overall eye displacement generally compensatory for body motion It was difficult to delineate the eye movements into fast and slow components. There were many saccade-like movements with velocities exceeding 100°/s but there were also eye movements intermediate in velocity between normal slow and fast phases (ie 50–100°/s) which were difficult to classify

Quantitative analysis of the vestibulo-ocular reflex

The gain and phase relationships for the vestibulo-ocular reflex response are illustrated in Fig. 3 The mean level of gain increased from 0.54 at 0.2 Hz to 0 90 at 1.3 Hz. Analysis of variance revealed a highly significant ($p<0\ 001$) increase in gain with frequency and also a highly significant difference between subjects The values of gain and phase were comparable to those obtained in previous experiments (Benson 1974 Hixson 1974 Barr et al 1976) for similar conditions

The directional preponderance here defined as the mean difference between slow phase eye velocity to the right and to the left in the vestibular response of these normal subjects was very small (mean over all frequencies = 0 08°/s) as was the standard deviation (S D = 1.88°/s) This reflects the symmetry of the response in normal subjects and provides a standard with which to compare the responses of patients with peripheral vestibular lesions (Barnes 1978)

Quantitative analysis of the cervico-ocular reflex

The responses of the cervico-ocular reflex were analysed in two ways First for all types of eye movement an analysis of the type described for the vestibular reflex was used to estimate the slow-phase velocity In this, all eye movements with a velocity greater than 100°/s were assumed to be saccades The gain and phase relationships are shown in Fig. 3

The slow phase component of the cervico-ocular reflex exhibited a very low gain in all subjects which, when meaned over frequency ranged from 0 02 to 0 14 There was no significant difference between the responses to different frequencies of stimulation a much larger inter-subject variability

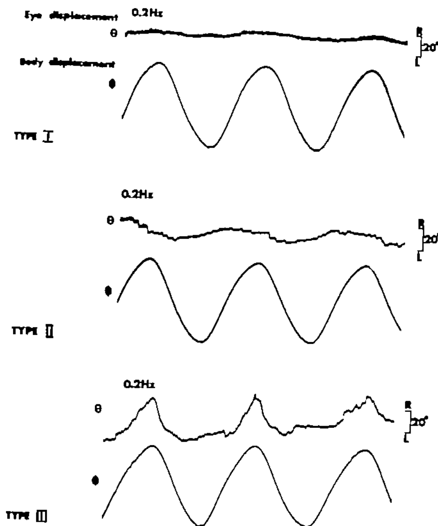


Fig 2 Examples of the Type I, II and III responses of the cervico-ocular reflex to angular oscillation of the body about the yaw axis with the head stationary

which has been noted previously (Barnes 1975 1976) The nett saccadic activity was apparently evenly distributed to both halves of the input sinewave although there were marked variations in the amplitude and timing of the saccades as is shown in Fig 1

Qualitative features of the cervico-ocular reflex

The oculomotor response resulting from neck torsion was by no means as stereotyped as that elicited from vestibular stimulation The examples in Fig 2 serve to illustrate the variety of responses which have been divided into three broad classifications In general each subject exhibited only one of the three types of eye movement throughout the range of stimulus frequencies

The Type I response (Fig 2 I) was the most common (7 out of 12 subjects) and was typified by small amplitude (±3 –4°) low velocity eye movements modulated in accord with the stimulus waveform but without saccadic activity Such small eye movements were elicited even when there was a substantial angular movement of the body with respect to the head This type of response is of a similar form to that described by Meiry (1966). In some of the Type I responses the slow phase eye movements were sparsely interspersed with saccadic movements having eye velocities ranging from 100–400°/s There appeared to be no repeatable pattern of saccadic activity as there was in the vestibulo-ocular response

The Type II response (illustrated in Fig 2 II) occurred in 3 subjects and was characterised by an overall eye displacement in which

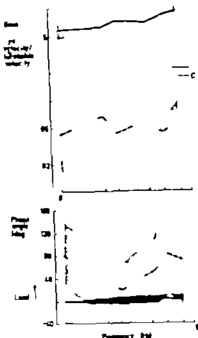


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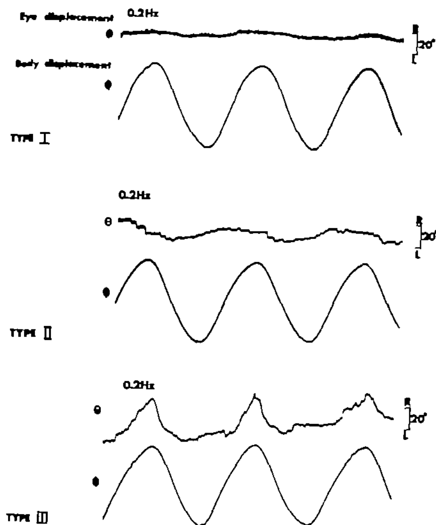


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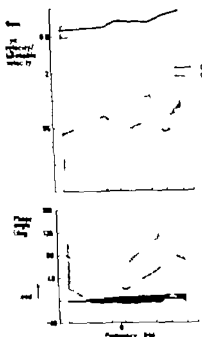


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The slow phase component of the cervico-ocular reflex exhibited a very low gain in all subjects which, when meaned over frequency, ranged from 0.02 to 0.14. There was no significant difference between the responses to different frequencies of stimulation, a much larger inter-subject variability

Table I *Gain and phase relationships between eye position and turntable position during sinusoidal oscillation of the body with respect to the head fixed in space Mean of 5 subjects who showed substantial overall eye displacement*

	Frequency (Hz)					
	0.2	0 4	0.5	0.8	1 0	1.3
Gain						
Mean	0 19	0.35	0.30	0.33	0.24	0.32
S D	0 13	0.29	0 20	0 16	0..3	0 26
Phase (deg)						
Mean	111.90	12 90	11.30	13 10	28 70	16.50
S D	73.50	54 10	38 40	37 60	86.30	63 70

being found than for the vestibular reflex The gain meaned over all subjects ranged from 0 041 to 0 065 and was comparable with values obtained by Meiry (1966) over the common frequency range and by Takemori & Suzuki (1971) at a frequency of 0 25 Hz

The phase relationship between body and eye movement was rather complex although for most subjects there was a large phase lead this is reflected by the mean values (Fig 3) which ranged from 21 to 80° The responses were thus not truly compensatory Inspection revealed that 4 of the subjects exhibited responses which were more closely compensatory with mean phase lead ranging from 4 to 26 but there was no significant difference in the gain of the reflex for these 4 subjects as compared with the remaining eight

The directional preponderance in the cervico-ocular response was very small and comparable in all subjects to that of the vestibulo-ocular reflex with an overall mean of −0 71 /s and a standard deviation of 1 38°/sec

The cervico-ocular responses have also been analysed by consideration of the overall angular displacement of the eye which for Types II and III responses was often considerable as is demonstrated by the examples in Fig 2 The same type of statistical analysis was used as for eye velocity that is a least squares error technique comp eye displacement with head displacement Only 5 of the subjects had significant eye displacement responses which were generally phase advanced with respect to head displacement The mean gain and phase relationships for these 5 subjects are presented in Table I

DISCUSSION

The vestibulo-ocular reflex

The response of the vestibulo-ocular reflex obtained in this experiment was very similar to that observed by other authors (Benson 1974 Hixson 1974) as demonstrated in Fig 1 The slightly lower values of the gain obtained here may be attributable to a different subject population but it is probable that two other factors are important

First a novel method of analysis was employed in which the whole of the response waveform was considered This would appear to be a less biased procedure than that normally used in which only peak slow-phase components are measured to obtain the gain and phase is estimated by crossover points of zero eye velocity This method of determining phase is particularly prone to error and the problem is exacerbated by the apparently non-linear nature of the slow-phase eye velocity Close study of waveforms has shown that even when the sinusoidal angular velocity of the head is provided by a servo controlled turntable and contains negligible distortion the slow-phase eye velocity exhibits more phase lag when measured relative to peaks rather than zeros Such distortion is averaged out by the regression procedure used here

The second important difference between this experiment and others lies in the nature of the experimental conditions It has been known for some time that the subject s level of arousal can modify the vestibulo-ocular response to a considerable extent (Collins et al 1961) In order to maintain a high level of arousal it has become customary to instruct

he subject to perform mental arithmetic during stimulation, particularly when the experiment is conducted with the eyes closed. We found that conducting the experiment in complete darkness with the eyes open was sufficient to maintain a high level of arousal as indicated by the level of saccadic activity in the response. However Barr et al. (1976) have recently shown that the gain of the vestibular reflex can be modified by the instruction set to the subject, the gain being close to unity when observing an imaginary stationary target and reduced when observing an imaginary target moving with the head. Subjects in our experiment were given no such explicit instructions but were simply told to stare blankly ahead in the dark. The wide variation in gain observed in our experiment and in those of other authors (Benson, 1974; Hixson, 1974) may reflect different modes of subject response with regard to imaginary target sources.

The cervico-ocular reflex

A recent review of the dynamic response to neck torsion has been made by Dichgans (1974) who concluded from the work of de Kleijn (1921), Grahe (1926) and Meiry (1966) that the effects of neck and labyrinthine stimulation on the oculomotor system were additive. There is clearly a large species difference, the reflex being potent in the rabbit but functionally inactive in the monkey (Dichgans, 1974) during passive head movements. Previous experiments on human subjects (Meiry, 1966; Takemori & Suzuki, 1971) indicate a gain decreasing from approximately 0.3 below 1 Hz to about 0.08 at frequencies above 0.4 Hz. Our results also indicate that if the slow-phase eye movements alone are considered the cervico-ocular reflex has a low gain (0.04–0.06) at frequencies between 0.2 and 1.3 Hz. Thus, the reflex would appear to be of little functional significance in assisting the process of ocular stabilisation during voluntary head movements (frequency approx. 0.5–2.0 Hz). Indeed, in many of the responses observed, the phase of the reflex was inappropriate for true compensation. However, there are two important points which need to be considered.

First, several of our subjects exhibited large overall displacements of the eye which were generally of a compensatory nature consisting of both slow and fast phase activity. Such eye movements cannot be dismissed as irrelevant. In a study of the rabbit cervico-ocular reflex, Isono (1955) classified 3 types of eye movement, namely slow, staircase and fast, which were not dissimilar to those described here. Likewise, Takemori & Suzuki (1971) reported that, *in human subjects, the eye* movements evoked by neck torsion were much larger with the eyes closed than with the eyes covered, suggesting some correlation with behavioural state. It is not inconceivable that the pendular eye movements observed in our experiment (Type III) may represent the effects of drowsiness which are known to produce such eye movements. Large eye deviations were not observed by Takemori & Suzuki (1971) or by ourselves when the subject was static, and the modulated nature of the response suggests that there may have been some entrainment with the stimulus.

The second proviso which must be made in the interpretation of the results is that, as far as voluntary head movements are concerned, the nature of the stimulus was not a natural one, particularly with regard to the degree of muscle tone. During a voluntary torsional movement of the head about the neck, any muscle spindle activity is likely to differ from that produced by an unresisted passive stimulus. However, in an attempt to simulate the influence of muscle tone by instructing the subject to resist the movement of the body, it was not possible to observe any difference in the cervico-ocular response.

The variability of the response to neck torsion suggests that the cervico-ocular reflex may be semi-redundant in human subjects with normally functioning labyrinths, at least for passively induced movements, although

it is difficult to assess its role during voluntary movements It is clear however that a reflex as observed functionally in newborn children is latent in the adult and can be potentiated in patients with vestibular lesions Frenzel (1928) was able to induce a nystagmus in response to torsion of the neck in patients with bilateral labyrinthine lesions and found that anaesthesia of neck muscles and joints reduced this nystagmus Further evidence of the partial recovery of head–eye coordination by the potentiation of the cervico-ocular reflex has been provided by Atkin & Bender (1968) and by Dichgans et al (1973) in the monkey However experiments by Barnes (1978) suggest that in subjects with bilateral labyrinthine loss the passively induced cervico-ocular response cannot always be elicited even though the patient exhibits good head–eye coordination during voluntary movements

In view of the evidence presented here and from the neurophysiological findings (e g Hikosaka & Maeda 1973 Rubin et al 1975) it appears probable that in normal humans the cervical afferent information serves to modulate the activity of second order vestibular neurons facilitating the action of the vestibulo-ocular reflex However the role of information from neck afferents seems likely to be important in other aspects of control (Abrahams & Rose 1975 Lindsay et al 1976 Wilson et al 1976) where afferent information from joints muscles and the visual cortex are all involved in monitoring and modifying system response in order to achieve optimum coordination of head eye and body movements

ZUSAMMENFASSUNG

Verglichen wurde die Okulomotorreaktion ohne Sehvermögen während zweier experimenteller Situationen in einer Gruppe von 1 normalen Menschen. Untersucht wurde *a*) die estibulo-okuläre Reaktion während einer Kopf und Korper betreffenden Oszillation auf einer Drehscheibe und *b*) die zerviko-okuläre Reaktion während einer Oszillation die nur den eigentlichen Korper betraf während der Kopf stillgehalten wurde Der Stimulus war eine sinusähnliche Oszillation (Spitzenwinkelgeschwindigkeit ±50 Grad/sec) mit Frequenzen zwischen 0,. und 0,3 Hz Die Augenbewegungen während der langsame Phase der vestibulo-okulären Reaktion glichen die Kei bewegungen aus und wiesen eine durchschnittlic mit der Frequenz ansteigende Erhöhung von 0,54-0 auf Bei der zerviko-okularen Reaktion dagegen wurde große Unterschiede festgestellt. Die Phase der langsam Augenbewegungen war von geringer Geschwindigke (durchschnittliche Erhöhung 0 05) und glich [illegible] al gemeinen die Körperbewegungen nicht aus. Während de Halsdrehung zeigten einige Versuchspersonen Sympto Großer totaler Augendeviationen (Schielen) die [illegible] Augenbewegungen der langsamen und schnellen Pha bestanden

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COMPARATIVE STUDY OF THE INFLUENCE OF AMINOGLYCOSIDE ANTIBIOTICS ON THE ACTIVITY OF THE HORIZONTAL SEMICIRCULAR CANAL IN THE FROG

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Abstract This work is an electrophysiological study made in the frog The technique allows one to test and to compare the actions of a number of aminoglycoside antibiotics directly introduced into the labyrinthic cavity on the spontaneous activity of a vestibular receptor—the horizontal semicircular canal The effects of aminoglycoside solutions have been compared with those of physiological solutions (NaCl 7 g/l Ringer) and of penicillin (not ototoxic) The results obtained show: (1) After the introduction of a physiological solution the activity disappears only very briefly (electrical artefact probably) after a few minutes the activity returns to its initial value A similar phenomenon is obtained with penicillin (2) When used at a dose of 10 μg all the aminoglycosides studied generally induced an important and lasting decrease in semicircular canal activity (3) These aminoglycosides have been classified according to their vestibular local toxicity Their descending order of influence is as follows: streptomycin dihydrostreptomycin amikacin (BBK 8) neomycin sisomycin gentamicin and lividomycin tobramycin kanendomycin (4) A parallel can be drawn between local vestibular toxicity and clinical ototoxicity The role and importance of the hemolabyrinthic barrier are noted and the notion of ototoxicity is discussed

It is well known that chronic injections of aminoglycoside antibiotics lead to auditory and equilibrium disorders Injuries of the inner ear induced by these antibiotics have been extensively described A number of studies (Hawkins & Lurie 1953 Vallancien 1955 Ardouin et al 1963 Wersäll & Flock 1964 Harada et al 1967) suggest that the histological damage may be preceded by functional troubles occurring as soon as the antibiotic comes into contact with the receptor: that is what we have intended to study

The electrophysiological method described by Gallais et al (in press) allowed us (1) to test the toxic action of various aminoglycosides upon one vestibular end organ (the horizontal semicircular canal) in the frog; (2) to make a comparative study of the action of these antibiotics

We have also compared the action of aminoglycoside solutions with that of physiological solutions (NaCl 7‰ and Ringer) and of penicillin G (antibiotic which is usually thought to be non-ototoxic)

The frog was chosen for various reasons previously specified (Gallais et al in press) but as it is well known that the semicircular canals are very similar among the vertebrates the conclusions drawn from this study are probably applicable to mammals in general and to man in particular

METHODS

In the frog the effects of the antibiotics or physiological solutions were tested on the spontaneous activity of the ampullary nerve of the horizontal semicircular canal The recordings were made on isolated head preparations (because the experiments are much easier in this condition) Each solution was studied in 11 to 20 preparations The technique used for opening the labyrinthic capsule and recording the activity of the ampullary nerve has been described previously (Caston et al 1977

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...allars et al. in press). The method of introduction of the solution into the inner ear was the same for all the compounds: 0.5 μl of the solution was injected into the labyrinthic cavity under paraffin oil (previously introduced in order to prevent nerves and membranous structures from drying) in contact with the horizontal canal ampulla by means of a microsyringe. This must be done without moving the electrode in order to compare the activity after introduction of the solution with the one recorded before introduction. This very small volume was selected in order to prevent the electrode from coming into contact with water.

Aminoglycosides (generally as sulphates) were in solution in NaCl 7 g/l. The 0.5 μl contained 10 μg of aminoglycoside sulphate or 10 μg of penicillin G (benzylpenicillinate sodium).

This dose was chosen because preliminary studies showed that it allowed comparisons to be made between the different drugs tested. Ten micrograms is the amount of streptomycin (most ototoxic antibiotic) for which the activity reappeared faintly after it had been completely suppressed. At that dose the graphs of all aminoglycosides are well spaced out between the graphs of streptomycin and of penicillin (Fig. 7); a smaller dose would not permit observation of significant differences between the various antibiotics.

In the following, we always use the words amount or dose of antibiotic and never concentration of the solution because the concentration at the receptor site cannot be estimated since neither the exact volume of the labyrinthic cavity nor the distribution of the drug in the perilymph, endolymph and various tissues are known.

The activity was recorded for 80 to 100 min: 15 min before injection of the solution (initial value) and 65 to 85 min after that injection. Measures were rarely done during a longer period of time as in most cases the spontaneous activity of the vestibular receptors recorded on isolated head preparations begins to decrease 2 or 3 hours after section of the head.

The number of spikes per second was computed every 5 min before the injection, every minute during the first 5 min after the injection, then every 5 or 10 min. The spontaneous frequency of the activity of the ampullary nerve of a canal can be very different from one frog to another (generally this frequency is 100 to 300 spikes/s), so in order to compare the action of different drugs we have in every frog expressed the activity observed after the injection as a percentage of the one recorded before the injection (conventionally fixed to 100). For each group of frogs we have drawn the curve of the mean spontaneous activity of the horizontal canal nerve and compared these mean curves.

The results were treated statistically: Mann and Whitney test (comparison between two groups), Kruskall and Wallis test (comparison between several groups) were used.

RESULTS

Effects of physiological solutions

NaCl 7 g/l pH 7.8. Twelve frogs (control group) were administered 0.5 μl NaCl 7 g/l into the labyrinthic cavity. Immediately after the introduction of the solution the activity was completely suppressed in most of the animals but it quickly increased and returned to a value not significantly different ($p = 0.05$) from its initial one within 5–10 min (Fig. 1).

NaCl 7 g/l pH 4.5. As the antibiotic solutions were acid (pH 4 to 6), we studied in 16 frogs the effect of a NaCl solution 7 g/l pH 4.5 in order to test the effect of pH on the ampullary nerve activity. pH 4.5 was obtained by adding a microdrop of sulphuric acid to the solution. Though the mean spontaneous activity remained slightly less when the pH was 4.5, the effect of NaCl 7 g/l pH 4.5 and that of NaCl 7 g/l pH 7.8 are not significantly different ($p = 0.05$) (Fig. 2).

Ringer. Fig. 2 shows that the mean curve (obtained from 15 frogs) is nearly the same

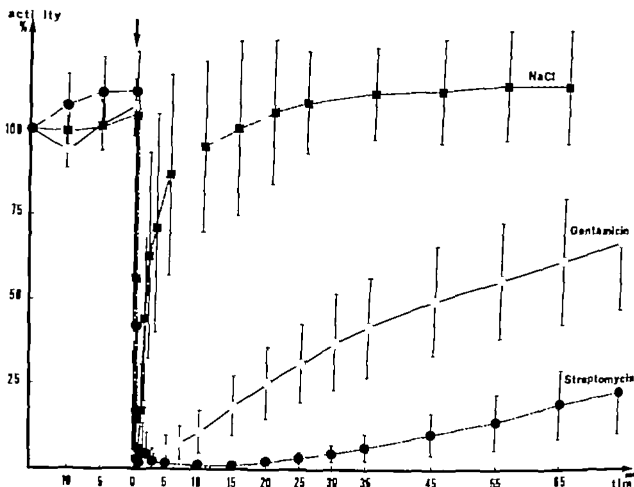


Fig 1 Influence of solutions of NaCl 7 g/l (pH 7.8) gentamicin and streptomycin on the spontaneous activity of the ampullary nerve of the horizontal semicircular canal *Abscissa* time in minutes *ordinat* activity recorded on the ampullary nerve expressed as percentage of its initial value (arbitrarily fixed at 100) Each point on the curves represents the mean activity recorded in 1 to 15 preparations At the arrow 0.5 µl of a NaCl 7 g/l solution or 0.5 µl containing 10 µg of gentamicin or streptomycin in solution in N Cl 7 g/l are introduced into the labyrinthic cavity Standard errors: $p=0.05$

as with NaCl pH 7 8 (there is no significant difference between the two curves)

Thus the introduction of any physiological solution into the labyrinthic cavity in contact with the horizontal canal ampulla induced a transient disturbance of vestibular spontaneous activity in all cases this activity was restored within 15 min

Effects of aminoglycoside antibiotics

Streptomycin We used streptomycin first because according to the literature it appears to be the most ototoxic antibiotic The effect was tested in 12 frogs In every case the antibiotic elicited a complete and almost immediate disappearance of the vestibular nervous activity this activity was suppressed for 15 to 45 min then it was gradually restored but 75 min after the introduction of the solution it was only 15 to 20% of its initial value (Fig 1)

Gentamicin We studied the effect of this antibiotic in 15 frogs In almost all of them the activity completely disappeared for 5 to 20 min after its introduction then the activity reappeared and gradually increased The mean value reached 75 min after the introduction of the antibiotic was near 70% Fig 1 shows that the restoration of the activity was much more rapid with gentamicin than with streptomycin the differences between the curves are significant ($p=0.05$) from the first minutes after the introduction of the antibiotics

Neomycin Twenty frogs were studied

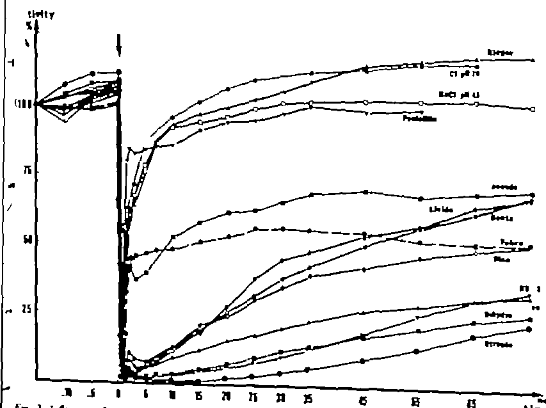


Fig. 2. Influence of several physiological solutions and antibiotics on the spontaneous activity of the ampullary nerve of the horizontal semicircular canal. *Abscissae, ordinate:* same as in caption to Fig. 1. Each point on the curves represents the mean activity recorded in 11 to 20 preparations. Standard errors have been omitted for clarity. At the arrow 0.5 μl of the physiological solutions or 0.5 μl containing 10 μg of antibiotics in solution in NaCl 7 g/l are introduced into the labyrinthic cavity.

From Fig. 2 it can be seen that the action of neomycin was less than that of streptomycin but greater than that of gentamicin. After its initial and sudden decrease, the activity was completely or almost completely abolished for 5 to 20 min (i.e., as long as with gentamicin) but it reappeared more slowly: as 75 min after the introduction of neomycin the mean activity had reached only one third of its initial value.

Dihydrostreptomycin. The ototoxicity of this antibiotic is also well known, but in man it is considered as toxic mainly for cochlear cells and to a lesser degree for vestibular receptors. We wanted to know whether its toxicity is the same as that of streptomycin or is much smaller when introduced direct into the labyrinthic cavity.

Seventeen frogs were studied. Fig. 2 shows that the restoration of the activity after its initial decrease was only slightly greater than in the case of streptomycin; nevertheless the differences between the two curves are significant except for the first 10 min and the last 10.

Other aminoglycosides. Some other aminoglycosides have been tested and their actions compared with those of the antibiotics mentioned above.

Lividomycin: the mean curve obtained from 11 frogs was very close to that of gentamicin (Fig. 2); the differences between the two curves are not significant ($p = 0.05$).

Sisomycin: its effect (studied in 16 frogs) was smaller than that of streptomycin, but greater than that of gentamicin (Fig. 2).

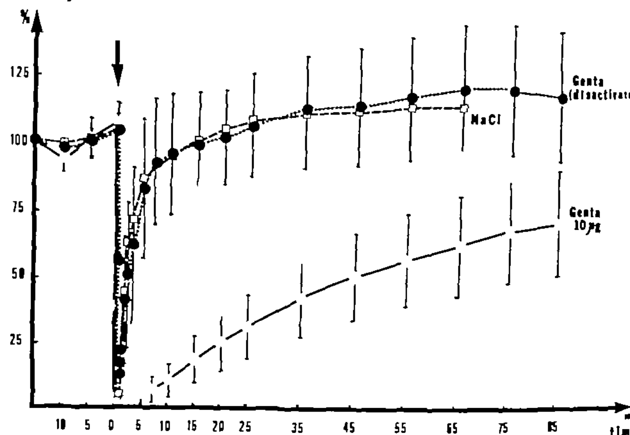


Fig 3 Comparison of the actions of NaCl 7 g/l gentamicin and *disactivated gentamicin* on the spontaneous activity of the ampullary nerve of the horizontal semicircular canal. *Absciss ordinate* as in Fig. 1 Each point on the curves represents the mean activity recorded in 1 to 15 preparations For NaCl standard errors have been omitted as they almost entirely overlap those of activated gentamicin. At the arrow 0.5 µl of a N 7 g/l solution or 0.5 µl containing 10 µg of gentamicin disactivated gentamicin are introduced into the labyrinth cavity

Amikacin (BBK 8) its effect was studied in 20 frogs For the first 45 min after its introduction into the labyrinthic cavity this antibiotic behaved as dihydrostreptomycin (the mean curves of the two aminoglycosides are almost identical) the amikacin curve then reached that of neomycin (Fig 2)

Kanendomycin its effect studied in 15 frogs was not so great as those of the above mentioned aminoglycosides Kanendomycin induced only a very brief decrease in activity (30 s to 2 min) The activity recovered 50% of its initial value in less than 10 min but 35 min after the introduction of the antibiotic the curve had nearly reached a steady level At the end of the experiment the value of the activity was 70% (no greater than that of gentamicin and lividomycin Fig 2)

Tobramycin its effect studied in 20 frogs was nearly the same as that of kanendomycin since the decrease in activity was general shorter than 2 min the activity recovered 50% of its initial value within 5 to 10 min after which it remained almost constant (Fig 7)

Effect of penicillin

Penicillin whose effect has been studied in 1 frogs gave results which were not significantly different from those of controls (the mean curve is very close to that of NaCl 7 g/l pH 4.5 Fig 2)

Effect of a disactivated aminoglycoside

We have used a disactivated aminoglycoside (i e without any antibacterial activity) the *N* penta acetylated gentamicin Its influence when tested on the horizontal semicircular canal activity in 12 frogs did not differ signifi-

cantly from that of the controls (the mean curve is nearly the same as that of NaCl 7 g/l pH 7.8 Fig. 3)

DISCUSSION

As we have seen in Results when any ionic solution is introduced into the labyrinthic cavity the activity recorded immediately drops and generally disappears. This seems to be an electrical phenomenon as the ionic solution contacts the electrode and impairment of ion exchanges between the solution and the cells probably occurs

With NaCl (7 g/l) *Ringer penicillin and* desactivated gentamicin these impairments are of very short duration so we may consider these solutions as non-ototoxic as for penicillin, Federspil et al (1976) drew similar conclusions

The results are not the same with aminoglycoside antibiotics as the activity is still low 10 min after introduction of the solution into the labyrinthic cavity: no activity (or nearly none) with streptomycin dihydrostreptomycin and amikacin 10% of the initial value of activity with the other aminoglycosides (except for kanendomycin and tobramycin with which the activity recovers 50% of its initial value) (Fig. 2)

Solutions of aminoglycoside antibiotics are acid (pH generally between 4 and 6) but results obtained show that the acidity is of no great importance since the activity recovers just as quickly with NaCl 7 g/l pH 4.5 as with NaCl 7 g/l pH 7.8

Other authors have found impairments of the activity of the labyrinthic or of the lateral line receptors after local application of these antibiotics. Wersäll & Flock (1964) on the microphonic output from the lateral line organ of *Lota* Tybergheim (1962) Feinmesser & Sohmer (1965) on cochlear potentials of the guinea pig, Harada et al (1967) on action potentials of the isolated posterior semicircular canal of the frog.

As we have seen the functional disorders induced by aminoglycoside antibiotics immediately after their introduction into the labyrinthic cavity are not the same as those induced by NaCl 7 g/l Ringer or penicillin neither are they due to acidity Hence they seem to depend on these antibiotics specifically We therefore studied the action of the aminoglycosides locally injected in order to make a comparison between them. For this comparison we consider (1) the slope of the curve which is steep or shallow according to whether the activity reappears and increases quickly or slowly after the rapid drop which follows the introduction of the antibiotic into *the inner ear cavity (2) the percentage of* activity that the semicircular canal recovers after a given time (for example 75 min after instillation of antibiotic)

According to the results that we have obtained we can classify the aminoglycosides studied in terms of their toxicity for the horizontal semicircular canal of the frog when they are introduced in contact with the receptor and used as the same dose This classification is as follows streptomycin > dihydrostreptomycin > amikacin (BBK 8) > neomycin > sisomicin > gentamicin ≈ lividomycin > tobramycin > kanendomycin

These aminoglycosides can be divided into three groups with respect to the criteria noted above

1 Streptomycin dihydrostreptomycin neomycin amikacin. the slopes of their curves are very shallow and after 75 min the activity recovers only one-third of its initial value sometimes less (streptomycin)

Gentamicin lividomycin sisomicin the slopes of their curves are pretty steep during the first 30 min but then the gradient decreases and the activity recovers after 75 min between 1/2 and 2/3 of its initial value

3 Kanendomycin and tobramycin the curves are at first steeply inclined, representing a quick recovery of the activity (50% of the initial value the activity disappearing only during the short-circuit) then the slope becomes much less steep moreover the activity

recovers 2/3 (kanendomycin) or 1/2 (tobramycin) of its initial value 75 min after instillation of the antibiotic

In the experimental conditions of our study the percentage of activity recovered by the receptor after introduction of the antibiotic is limited by the absence of blood flow (the latter would make for elimination of the antibiotic) and by the comparatively short time during which measurements are made (at most 80 to 90 min from instillation of the antibiotic into the ear) therefore it may be that the effect of a given dose of an antibiotic on the semicircular canal activity is greater in our experiments than it would be in intact animals

It may be noted that local toxicity of an antibiotic is related to its chemical structure the molecules of streptomycin and of dihydrostreptomycin have nearly the same structure and their local toxicity does not differ greatly The same is true of gentamicin and sisomicin In both cases the second molecule is obtained by hydrogenation or dehydrogenation of the first one On the other hand we may be surprised by the differences in toxicity between kanendomycin and amikacin as only a radical is different between the two molecules But in fact adding an additional radical to a molecule is very important since penta acetylated gentamicin has lost its antibiotic properties and is without any toxicity in our experimental conditions thus it seems that the presence of free NH_2 radicals in this molecule is important for its vestibular toxicity

Let us compare our results with those of Harada et al (1967) regarding also a semicircular canal of the frog These authors noted as we did that (i) antibiotics act immediately after their injection (ii) streptomycin is the most active aminoglycoside and (iii) the action of dihydrostreptomycin is significantly weaker than that of streptomycin However these authors found that the actions of kanamycin and neomycin are nearly identical whereas in our experiments it appears that kanamycin is not so toxic the discrepancy between their results and ours may be d to the fact that we used kanendomycin (i e kanamycin B) whereas according to th statement they used kanamycin (i e alm exclusively kanamycin A) Moreover th conditions of the experiments were not iden cal as these authors worked *in vitro* while w worked on receptors *in situ* thus they cou remove the antibiotic from the bathing so tion and study the reversibility of its actio whereas we could not We do not know ex actly what happens after a substance has be introduced into the ear from the perilym and/or the endolymph it probably spread to all the labyrinthic receptors and perha outside the ear But in any case it cannot eliminated by the blood flow In other respec in our experiments the receptors are still co nected to the brain which influences the ve tibular receptors by means of the efferent ve tibular system (Gribenski & Caston 197 it can be surmised that this system enhances reduces the disorders due to the action aminoglycosides on the vestibular recepto

CONCLUSION

When intramuscular or intravenous inj tion of a drug does not produce auditory vestibular disorders the drug is said to non-ototoxic We can refer to this kind ototoxicity as *clinical ototoxicity* On t other hand it appears from this study th an aminoglycoside antibiotic can impair t function of a vestibular receptor when intr duced into the labyrinthic cavity we can re to this kind of ototoxicity as *local ototoxici*

A drug having no local ototoxicity is u likely to have clinical ototoxicity such is t case with penicillin (Stupp et al 1973) On t contrary a drug having local ototoxicity m have strong (e g streptomycin) or weak (e amikacin Brummet & Fox 1977 Federspil Schätzle 1977) clinical ototoxicity depend on how much and how quickly the drug pe trates and accumulates in the labyrinth cavity (as is known ototoxicity of amir

oglycosides is due to retention of these antibiotics in the labyrinthic fluids. Voldrich 1965 Stupp et al 1966 1973 Meyer zum Gottesberge & Stupp 1969 Von Ilberg et al 1971 Quante et al. 1974 Federspil et al 1976 1977) This in turn depends on two conditions (1) The drug can or cannot pass through the hemolabyrinthic barrier in the first case the drug will accumulate in the labyrinthic fluids more especially when its blood concentration is high (this is conditioned by the frequency and the number of the injections) (2) The drug is either quickly excreted through the kidneys or else it is not (If it is then only small concentrations of it will be found in the perilymph and endolymph even if the drug can pass through the hemolabyrinthic barrier and clinical ototoxicity will probably not occur) That might explain the discrepancy which appears between local and clinical ototoxicities with dihydrostreptomycin and amikacin (whereas all other aminoglycoside antibiotics studied show a close correlation between these two kinds of ototoxicity) Moreover whereas local ototoxicity is probably similar in frog and in man since as already said organization and functioning of the peripheral vestibular apparatus are similar this does not hold good for clinical ototoxicity clinical ototoxicity of any aminoglycoside antibiotic is likely to be very different in frog and in man for between them important differences exist with regard to hemolabyrinthic barrier structure and function of kidneys and metabolism of drugs

Therefore even for clinical use it is important to know whether a drug has local ototoxicity or not in fact, a drug which is ototoxic when introduced into the inner ear though usually not ototoxic from a clinical point of view can be clinically ototoxic in special circumstances renal failure (Wersall et al 1969) disorders of the permeability of the hemolabyrinthic barrier and/or of kidney functioning due to simultaneous administration of another drug (i.e furosemide or ethacrynic

The method used in this study enables us to decide rapidly and with a very small quantity of a drug whether the drug in question has local ototoxicity or not and if it has to compare its local ototoxicity with that of a number of well-known aminoglycoside antibiotics Such a comparison would be convenient when such a drug has to be administered for clinical purposes

RÉSUMÉ

Ce travail est une étude électrophysiologique permettant de tester et de comparer chez la Grenouille l'action de divers aminoglycosides, introduit directement dans la cavité labyrinthique sur l'activité spontanée d'un récepteur vestibulaire le canal semi-circulaire horizontal Les actions des solutions d'aminoglycosides ont été comparées à celles de solutions physiologiques (Nacl 7 g/l Ringer) et la pénicilline (non ototoxique) Les résultats obtenus montrent que 1 L'introduction d'une solution physiologique ne provoque qu'une chute d'activité de très courte durée (artefact électrique probablement); après quelques minutes l'activité retrouve sa valeur initiale Il en est de même avec la pénicilline 2° Tous les aminoglycosides étudiés provoquent, à la dose employée (10 µg), une altération plus ou moins marquée et plus ou moins durable de l'activité du canal semi-circulaire 3° Un classement de ces aminoglycosides a pu être établi en fonction de leur toxicité vestibulaire locale Dans l'ordre d'influence décroissante nous trouvons la streptomycine, la dihydrostreptomycine, l'amikacine (BBK 8), la neomycine la sisomycine la gentamicine et la lividomycine la tobramycine la kanamycine 4 Un certain parallélisme peut être établi entre la toxicité vestibulaire locale et la toxicité clinique. Le rôle et l'importance de la barrière hémolabyrinthique sont indiqués et la notion d'ototoxicité est discutée

ZUSAMMENFASSUNG

Diese Untersuchung ist ein elektrophysiologisches Studium zur Prüfung der T xizität von Aminoglycosiden auf die Spontanaktivität eines vestibulären Endorgans (horizontaler Bogengang). Die Aminoglycosiden wurden in die Labyrinthhöhlung von Laubfröschen gerade hereingeführt Sie wurden mit physiologischen Lösungen (0 7% NaCl Ringer) und mit Penicillin G (ohne Ototoxizität) verglichen Die Ergebnisse zeigen. 1) Wenn eine physiologische Lösung oder Penicillin hereingeführt ist, zeigt sich ein kurzer Aktivitätsabfall (elektrisches Artefakt wahrscheinlich) Nach wenigen Minuten erreicht die Aktivität wieder den anfänglichen Wert 2) Bei Verwendung der Dosis von 10 µg verursachen die studierten Aminoglycosiden im allgemeinen eine wichtige und andauernde Abnahme der semizirkulären Kanalaktivität. Sie ist mehr oder weniger stark und lang 3) Wir haben die Aminoglycosidordnung nach lokaler vestibulärer Toxizität

eingesetzt In abnehmender Ordnung finden wir Streptomycin Dihydrostreptomycin Amikacin Neomycin Sisomycin Genta und Lividomycin Tobramycin Kanendomycin 4) Es kann eine Parallele gezogen werden zwischen lokaler Ototoxizität und klinischer Ototoxizität Die Funktion und die Bedeutung der hämolabyrinthischen Grenze wird gezeigt Die Frage der Ototoxizität wird diskutiert

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CALORIC PATTERN TEST WITH SPECIAL REFERENCE TO FAILURE OF FIXATION–SUPPRESSION

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Abstract Fixation–suppression of caloric nystagmus was studied in 1230 consecutive cases. An analysis of caloric nystagmus with failure of fixation–suppression (FFS) made it possible to classify patterns of FFS into three types Type I FFS was observed bilaterally in caloric nystagmus. Type II FFS was recognized unilaterally on either side Type III FFS was seen in either direction of nystagmus. On the basis of these three types the underlying neuroanatomical substrate responsible for FFS is discussed

Ocular fixation of vestibular nystagmus as the junction of the visual and the vestibular systems has been used clinically with increasing experimental findings (Takemori & Cohen 1974)

We standardized the effects of eye opening and ocular fixation on caloric nystagmus (Kato et al. 1977) and have utilized this procedure as a routine test since 1974 In the present paper we review the significance of this test reporting cases with failure of fixation–suppression of caloric nystagmus (FFS) (Coats 1970)

MATERIALS AND METHODS

The subjects were 1289 patients who visited the Electronystagmography (ENG) Laboratory and 29 others who were admitted to the Neurosurgical Division with lesions documented by objective evidence Of 1318 patients 88 patients were excluded 30 were postoperative cases 51 had incomplete clinical or ENG data 4 were taking either diphenylhydantoin or barbiturates 3 had congenital nystagmus. Of the remaining 1230 patients 122 showed FFS in the caloric test (Table I) (1) and (2) contained cases with lesions documented by roentgenographical operative or post-mortem evidence Patients in (3) had one or more ENG abnormalities indicative of brainstem or cerebellar lesions (Coats 1970) and clinical evidence of central nervous system disorder Cases with organic mercurial intoxication had been diagnosed as having Minamata disease by the staff of Niigata University (Mizukoshi et al 1975 Ino & Mizukoshi 1977) Degenerative diseases were divided into three clinical groups (Baloh Kon-

Table I *Incidence of FFS in brain lesions at different locations*

	FFS
(1) Cerebrum	
Tumor	2/8
Vascular	1/4
(2) Brainstem & cerebellar tumor	
Brainstem tumor (intrinsic)	1/3
Brainstem tumor (extrinsic)	1/2
Cerebellopontine angle tumor	6/13
Cerebellar tumor	5/7
(3) Brainstem & cerebellum	
Vascular	23/34
Head injury	9/18
Brainstem and/or cerebellar disorders	17/31
Basilar impression	1/1
(4) Organic mercurial intoxication	32/87
Degenerative or inflammatory	4/32

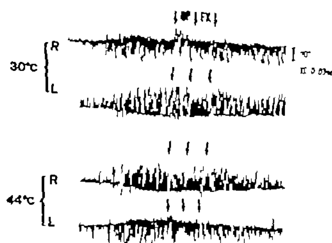


Fig 1 ENG recordings in a typical case of Type I The effects of OP and FX upon slow-phase velocity cannot be observed in both nystagmus directions

rad & Honrubia 1975) 12 cases with clinically demonstrated cerebellar atrophy (group I) 9 with brainstem plus cerebellar atrophy (group II) 4 with Friedreich ataxia and 2 with spastic spinal paraplegia (group III) In addition one case with progressive multifocal leukoenceph alopathy (PML) and 4 cases with brainstem encephalitis were included

Recording procedure

Eye movements were recorded with a Sanei ENG apparatus (4 channels) with a rectilinear pen linkage

Electrodes were placed lateral to the outer canthi to record horizontal eye movements and above and below the right eye to record vertical eye movements A ground electrode was located on the forehead

Test procedure

ENG examination was carried out with the eyes closed in a dimly illuminated room

To test the effect of eye opening (OP) and ocular fixation (FX) upon caloric nystagmus the caloric test was modified as follows Patients were requested to open their eyes 55 sec after the onset of irrigation for 10 sec at which time the eyes were covered with thick white paper at a distance of about 10 cm (Figs 1–4 OP) Then they were asked to fix gaze on the examiner s index finger held about 30 cm away during the following 10 sec (Figs 1–4 FX) Mean maximum intensity of slow-phase eye velocity was determined during the control period from 50–55 sec after the onset of irrigation The slow-phase velocity induced by OP and FX was obtained during the first half of each 10-sec period and compared with the control period Alertness was maintained by mental arithmetic throughout the caloric test

Details of the caloric test and calculation of the percentage reduction in slow phase velocity were described previously (Kato et al 1977)

Cases with less than 20% reduction in slow phase velocity of caloric nystagmus by OP were referred to as FVS (Failure of Visual Suppression of Caloric Nystagmus) Cases with less than 50% reduction induced by FX were referred to as FFS (Failure of Fixation Suppression of Caloric Nystagmus) (Coats 1970) The experimental results will be presented only for FFS in order to avoid confusion with FVS as will be described in the discussion

If the paper speed of the ENG recording runs at 0 3 cm/sec the degree of fixation-

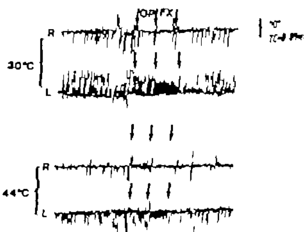


Fig 2 A representative case of Type II The effects of OP and FX are not observed when caloric stimuli are applied to the non-affected ear irrespective of the water temperature

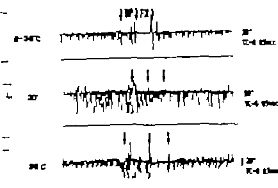


Fig. 3. FFS is related to the growth and reduction of tumor in a patient with postoperative recurrence of acoustic neurinoma. A follow-up study using ENG shows negative FFS (the upper trace), which is later followed by positive FFS (the second trace) on admission. Postoperative examination (the third trace) shows FVS (see Discussion) but not FFS.

suppression in slow-phase velocity can be evaluated as a pattern, which we have called the caloric pattern test.

RESULTS

Of 1230 cases, 122 showed FFS (Table I). Of the cases with FFS, 77 were subjected to the alternate cold and hot caloric test at water temperatures of 30°C and 44°C, and could be classified as follows.

Type I

FFS was observed bilaterally in caloric nystagmus. Diffuse involvement of the central nervous system such as degenerative or inflammatory diseases and organic *mercurial intoxication* were recognized.

Type II

FFS was seen unilaterally on either side regardless of the water temperature applied as caloric stimuli. Cerebellopontine angle syndromes such as acoustic tumor, meningioma, and epidermoid were included.

Type III

FFS was recognized in either direction of nystagmus depending upon the water temperature used as caloric stimuli. Vascular disorders, head trauma, and unilateral tumors of the cerebellar hemisphere were included.

Now typical cases of ENG for Types I, II and III will be presented.

Fig. 1, a representative of Type I, shows *caloric nystagmus* in a case with olivo-ponto-cerebellar atrophy.

Caloric nystagmus is shown only by derived curves. *Upward deflections in the ENG* represent eye movements to the right. Upper two traces are caloric nystagmus induced by water at 30°C, right (R) and left (L) ear stimulation respectively. Lower two traces are nystagmus induced by water at 44°C, right (R) and left (L) ear stimulation respectively. The period of OP and FX during maximum intensity of caloric nystagmus is indicated by vertical arrows. Bottom trace is time base of 1 mark/sec. Calibration and time constant (TC) are indicated on the upper right in most of the traces. The following Figures 2 to 4 use the same abbreviations and test conditions as does Fig. 1.

As can be seen from Fig. 1, effects of FX were not observed in both nystagmus directions and nystagmus frequency was increased during FX.

The ENG shown in Fig. 2 was recorded from a case of tentorial meningioma at the

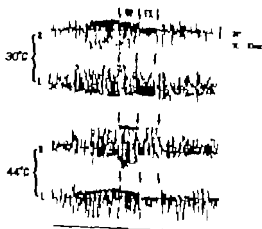


Fig. 4. A representative case of Type III. FVS and FFS are observed only in caloric nystagmus to the right.

taken as positive in calculation FFS was observed in Type I or Type III

DISCUSSION

When subjects are induced to fix gaze during the elicitation of caloric nystagmus nystagmus is not suppressed and is sometimes activated This is called failure of fixation–suppression of caloric nystagmus (Coats 1970) paradoxical caloric response (Maccario Backman & Korein 1972) or ocular fixation reversal phenomenon (Torok 1973) and is regarded as a sign of central ENG abnormality (Coats 1970)

Coats (1970) maintained that FFS was found in patients with brainstem and cerebellar lesions rather than in those with cerebral lesions

In our cases FFS tended to be found in those with brainstem or cerebellar lesions However 3 of 12 cases of cerebral lesion were suspected of FFS so that it is not clear whether lesions of the brainstem and the cerebellum are the only neuronal substrates required in FFS

In normal subjects there was a significant difference in percentage reduction of slow phase velocity under the test conditions for FVS and those for FFS (Kato et al 1977)

This led us to assume that there might be a difference in the range of defense between the two tests

Studies of many cases up to the present time show

(1) When FFS is positive FVS is also certainly positive and never becomes negative

(2) In cases in which FFS is positive in preoperative examinations of tumor of the cerebellar hemisphere acoustic tumor and so forth it turns out to be dramatically negative in postoperative examinations but FVS remains positive

(3) FVS is observed infrequently in cases of cerebral lesion

However examination of FVS is at times difficult to judge because the percentage reduction in slow-phase velocity is small Probably nystagmus is inhibited due to the ey closure (Tjernström 1973) and may be re leased when patients are asked to open their eyes and are placed under conditions for FVS Therefore examination of FFS alone may be sufficient

As to the neuroanatomic substrate re sponsible for FFS there are still many un known points In animal experiments loss of visual suppression (VS) of caloric nystagmus was produced after flocculus lesions (Take mori & Cohen 1974) At that time loss of VS was observed in the nystagmus direction toward the lesion site

When our cases are compared with data ob tained from the animal experiment FFS is ob served bilaterally in Type I It is found in cases with cerebellar degeneration acute in toxication of diphenylhydantoin (not pre sented in this paper) and organic mercurial intoxication This suggests bilateral diffuse in volvement of the central nervous system

That FFS is observed in either direction of nystagmus depending upon caloric stimuli as represented in Type III can also be explained by the experimental data It is Type II that comes into question When caloric stimuli are applied to the non affected ear caloric nystagmus under cold stimulus takes a direc tion opposite to that under hot stimulus and vice versa (Fig 2)

Why is FFS observed only when caloric stimuli are applied to the non-affected ear? Ac cording to Kato et al (1976) and Mizukoshi et al (1976) FFS was observed with high in cidence when the acoustic tumor was in Stag III chronologically (Cushing 1917) at a tim when abnormal ENG findings were observe in all cases showing that the disease was con siderably advanced

Is it not possible to assume that the in fluence of the tumor has extended to the non affected side on the basis of ENG findings?

Presumably Type II may be a subdivisio of Type I and FFS ought to be observed bi laterally But FFS on the affected side is cu off due to severe hypoexcita... of the ves

bular function on that side and consequently FFS might be noted only on the non-affected side.

Nevertheless, the fact that FFS is observed in caloric nystagmus of which direction differs in the alternate cold and hot caloric test can not fully be explained by the animal experiments alone.

As shown in Table I, FFS was definitely recognized in cases with brainstem lesion, which was also confirmed by Coats (1970), Demanez & Ledoux (1970) and Alpert (1974). Hence the neuroanatomic substrate in the brainstem related to FFS is the crucial point to be discussed. The flocculus receives visual signals through a climbing fiber pathway via the inferior olive (Brodal 1940; Maekawa & Simpson 1973) and through a mossy fiber pathway presumably via the superior colliculus (Maekawa & Takeda 1975). A recent animal experiment has revealed that the lateral pretecto-olivo-floccular pathway (Maekawa & Simpson 1973) has no relevance to the immediate modification of the vestibulo-ocular reflex by visual stimuli. A mossy fiber pathway via the superior colliculus is related to the immediate adjustment of vestibulo-ocular reflex by visual stimuli (Kato et al., in press). These experimental results are well compatible with the present clinical studies that neither FFS nor reduced amounts of fixation–suppression were observed even in a case of a pontine glioma large enough to cover almost the entire inferior olive (Fig. 6). While in a case of midbrain tumor, FFS was evident. Furthermore, a very recent animal experiment has shown that the reticular tegmental pontine nucleus conveys visual signals to the flocculus through a mossy fiber pathway (Kawasaki et al. 1978; Takeda & Maekawa 1978). Therefore with the advance of studies relevant to fixation–suppression of caloric nystagmus in the brainstem, the three types of FFS described could be clearly delineated.

It is hoped that the caloric pattern test will be used routinely to detect lesions in the brainstem and the cerebellum.

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ZUSAMMENFASSUNG

Bei 1230 Patienten wurde der Effekt der Okularfixation auf kalorischen Nystagmus während der maximalen Winkelgeschwindigkeit der langsamen Phase untersucht. Von der Analyse des kalorischen Nystagmus mit FFS (failure of fixation–suppression of caloric nystagmus) aus konnte die Form des FFS in folgende 3 Typen klassifiziert werden: Typus I. FFS wurde in bilateral-erzeugtem kalorischem Nystagmus beobachtet. Typus II. FFS wurde unilateral in jeder Seite beobachtet. Typus III. FFS wurde in einer bestimmten Richtung des kalorischen Nystagmus beobachtet. Von diesen 3 Typen wurde unterliegende neuroanatomische Struktur für FFS diskutiert.

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THE INFLUENCE OF PNEUMATIZATION OF MASTOID BONE ON CALORIC NYSTAGMUS RESPONSE

A Clinical Study and a Mathematical Model

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Abstract Pursuing the problem whether and why the pneumatization of mastoid bone has any influence on caloric nystagmus, we examined 15 large or extensively (L P) and 15 poorly (P.P) pneumatized subjects. Following water irrigation (44° and 30°C, 30 sec) we checked the parameters maximum SPV max. frequency latency duration and time of max. response by ENG recordings. Both groups differed significantly for all parameters except duration ($p<0.001$). On comparing these findings with an earlier proposed model (Bock & Bromm, 1977) we could show good correspondence for the P P group by doubling the parameter 'thermal diffusivity of mastoid bone' An even better correspondence could be found by diminishing the parameter coefficient of temperature changes by perfusion The theoretical and practical implications and conclusions of our findings are discussed

Slight differences of temperature pass quickly through the mastoid bone (Frenzel 1925 Dohlman 1925) An extensively developed pneumatization of mastoid bone could therefore act as a better heat insulation than a poor pneumatization In view of this consideration we tried to answer two questions. (1) Is there a relationship between the pneumatization of the mastoid bone and the degree of caloric response? (2) Could we explain our results by using a mathematical model of caloric nystagmus

METHODS

In cooperation with the E.N T Clinic of the University of Hamburg we examined 15 subjects with extensive or large pneumatization (L.P) and 15 subjects with poor pneumatization (P P) on either one or both sides None of them had a history of vertigo or vestibular attacks By using roentgenograms (position of Schueller) we were able to decide the degree of pneumatization of the mastoid bone following Diamant (1940) we evaluated a smaller area than 6 cm² in this projection as P P Every subject underwent a caloric test using 30 cm³ hot and cold water (44 and 30°C) for 30 sec This test was performed in darkness with eyes closed while the subjects had to solve simple mathematical tasks to confirm a high level of vigilance Time intervals between individual calorizations were 20 min. Before and after each test we checked the calibration. Nystagmus measuring was performed by ENG recordings using an 8-channel EEG amplifier with paper writer (Fa. Schwarzer) The bandwidth of the whole system ranged between 0 and 50 Hz. The caloric response was calculated from the ENG recordings by checking five parameters latency duration time of maximum response maximum slow phase velocity (SPV) and maximum frequency during the interval of culmination (15 seconds)

Clinical study

Table 1 and Fig. 1 summarize the main results Highly significant differences ($p<0.001$) between the two groups are to be seen. The maximum SPV in the P P group amounts to nearly twice the value of the L.P group and the maximum frequency has a significantly higher level as well The time of maximum

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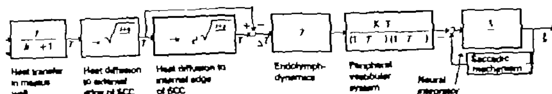
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ig 2 Mathematical model of caloric nystagmus as proposed by Bock & Broeren (1977)

Model parameters

Heat transfer coefficient $k = 1$ sec (during irrigation) or 62 sec (else).

Blood perfusion coefficient $l = 0.008$ sec^{-1}

Thermal diffusivity of vacuolized part of petrous bone $= 0.0016$ cm²/sec

Thermal diffusivity of compact part of petrous bone $= 0.016$ cm²/sec.

Distance meatus–external SCC edge $= 0.6$ cm.

Distance meatus–inner SCC edge $= 1$ cm.

Vestibular system gain $K = 5$

SCC long time constant $T = 8$ sec.

Time constant of mechanoneural transmission $T = 100$ sec.

Is the Laplace operator

changes caused by blood perfusion' to $l' = 0.004$ sec in addition to a changed thermal diffusivity $a = 0.0034$ cm²/sec (curve 3)

DISCUSSION

Our clinical results show a highly significant difference between P P and L P ears This difference is in respect to time course and intensity of the caloric response All values for the L P group agree closely with earlier findings by Henriksson (1956) Hamersma (1957) Pfaltz (1957) Jongkees (1953) Hinchcliffe (1967) We were able to calculate a similar result from experimental data given by Reker (1977) in 3 subjects with P P According to this calculation there is a significant difference in the caloric response (see Table III) when stimulating with 10°C water Although we could not find any other papers with quantified data, Frenzel (1925) wrote in his "Beiträge zur Theorie und Methodik der thermischen Vestibularerregung: daß die Ausbreitung des Temperaturgefälles auf dem Knochenwege und nicht via Trommelfell-Paukenluft erfolgt. Diese Knochenzuleitung hat eine luftumgebende Zirkumferenz und ist kompakt. " In the same year Dohlman (1925) noted. "Hieraus wäre zu schließen, daß die Wärmeleitung von der Verteilung zwischen Knochen und lufthaltigen Räumen abhängig sein muß " Slight differences in temperature pass very quickly through the mas-

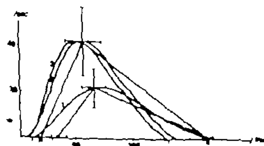


Fig 3 Nystagmus slow phase velocity as predicted by the model(large or extensive (1) poor (2), $= 0.0040$ poor (3), $= 0.0034$ l 0.004 Experimental values L P (●) and P P (■) including standard deviations

Table III *Maximum slow phase velocity (degrees per second) and standard deviations (in parentheses)*

Calculated from U Reker 1977 (*Arch Oto-Rhino-Laryngol* 14 247–256, Table I)

Temperature (100 cm³ 15 sec)	Pneumatization: Poor (=3)	Extended or medium (=22)	
30°C	14.2 (± 7 4)	13.0 (±4 6)	s.
20°C	28 4 (±15.5)	25.3 (±8 0)	n.s.
10°C	47 8 (±16 1)	31 2 (±7 1)	$p<0.01$

Table I *Caloric responses (mean values standard deviation in parentheses) in subjects with poor pneumatization (n=15) compared with subjects with large or extensive pneumatization (n=15)*

	Poor	Large or extensive	
Slow phase velocity (degree sec)	42 1 (±14.8)	21 8 (±8 7)	$p<0.001$
Frequency (sec)	2.59 (±0 90)	2 07 (±0 06)	$p<0.001$
Time of maximum response (sec)	57 0 (±16 1)	66 1 (±10 7)	$p<0.001$
Latency (sec)	19 9 (±9.2)	24.8 (±6.5)	$p<0.01$
Duration (sec)	146.8 (±31 7)	143.8 (±34.5)	$p<0.05$

response and latency is significantly earlier for the P P group than for L P group The whole caloric response lasts longer for the P P group ($p<0.05$) On the whole the caloric response of the P P group appears to be both stronger and earlier

As we can see from Table II there are similar results when comparing the caloric response in those 7 subjects with P P on one side only These intra individual differences are highly significant ($p<0.001$)

Mathematical model

As shown in Tables I and II these differences are presumed to be caused by the different thermal diffusivity of the mastoid bone in the two groups To check this assumption we performed calculations using a mathematical model of caloric nystagmus The model used here (Fig 2) was analysed in detail in a previous paper (Bock & Bromm 1977) It consists of a series of differential equations, describing the thermal properties of the mastoid bone and the vestibular reaction to the thermal gradient across the lateral semicircular canal Model output is eye position according to the ENG signal derived from human subjects Compared with the original data of Bock & Bromm (1977) parameter values (a specification for them is given in the caption to Fig. 3) were slightly altered to adapt the model to our present clinical results of clinically healthy subjects Curve 1 in Fig 3 shows nystagmus SPV as predicted by the model To allow a better comparison with the clinical results data from Table I are also plotted in Fig 3 Curve 2 is obtained by changing the parameter thermal diffusivity from $a = 0.0016$ cm²/sec to $a = 0.0040$ cm²/sec Now there is a rather good correspondence with clinical results of P P ears An even better match can be achieved by diminishing the parameter 'heat

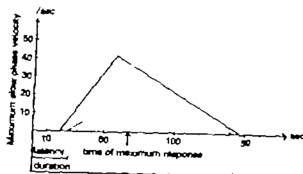


Fig *1* Caloric response in subjects with poor (—) $n=15$ pneumatization and a large/extensive one ( ) $n=15$ as calculated from Table I

Table II *Differences of caloric responses in 7 subjects with poor pneumatization on one side and large/extensive pneumatization on the other*

	Mean value	Standard deviation	
Slow phase velocity (degree sec)	17.4	±11 2	$p<0.001$
Frequency (sec)	1.25	± 0 47	$p<0.001$
Time of maximum response (sec)	16 6	± 9.3	$p<0.001$
Latency (sec)	9 6	± 5 1	$p<0.001$
Duration (sec)	33 1	±44 4	$p<0.05$

wenn der Parameter „Wärmeleitung durch das Felsenbein" etwa verdoppelt wird. Eine noch bessere Anpassung ergibt sich, wenn der „Koeffizient für durchblutungsbedingte Temperaturänderungen" verkleinert wird. Die theoretischen und praktischen Implikationen und Folgerungen dieser Ergebnisse werden diskutiert.

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toid bone An extensively developed pneumatization of mastoid bone could therefore act as a better heat insulation as Maurer noted in 1953 that a caloric reaction could be released from an extensive or large pneumatized mastoid only by very strong stimulation and with very low intensity and long latency respectively rather than by thermal stimuli through the meatus acusticus *externus*

From our clinical findings we could assume that the difference between the two groups could be caused by a differing thermal diffusivity of the mastoid bone If this assumption were true the response of a mathematical model of caloric nystagmus would fit the caloric test results of the L P and P P ears as well This should depend on the value of the parameter heat diffusivity of mastoid bone while leaving all the other model parameters unchanged

Theoretical predictions of caloric nystagmus are all within the ±1 S D range of clinical values except for duration There is a particularly good fit for slow phase velocity in the cumulation interval and time of maximum SPV which are more reliable parameters of caloric nystagmus

Because the only change made in the model to predict curve 2 instead of curve 1 was to increase the value of *a* (thermal diffusivity of mastoid bone) by the factor 2 5 our results support the assumption that the different vestibular reaction in P P ears is caused by a greater thermal diffusivity *a* We could not find any reliable measurements of *a* in bones having differing degrees of pneumatization Nevertheless the above assumption of a change by a factor of 2 5 appears reasonable Because *a* of air is about 1/20 the value of pure bone it can be assumed that heat transport takes place predominantly through the bone In the cross-section of a temporal bone with poor pneumatization there is more bone and less air per area and consequently the heat can be transported more easily In addition the length of the heat path curving along the walls of air cells is diminished as the air cells are smaller thus improving the heat transport potential of the bone

Furthermore our theoretical results suggest that blood Perfusion *Γ* might be reduced in ears with P P which can be explained by a reduction of the well perfused mucosa of air cells

Summarizing our clinical and theoretical results respectively we would conclude that ears with poor pneumatization of the mastoid bone might have a better heat transmission to the horizontal semicircular canal than ears with an extensively developed pneumatization As we can see from the mathematical model of caloric nystagmus this might be due not only to the degree of pneumatization but also to an altered vascularization

When stimulating with a caloric test we find another response as usually formulated. A nystagmus of shorter latency stronger intensity and reaching the time of maximum intensity faster Therefore we propose a careful examination of the pneumatization of the mastoid bone in ears with a very strong caloric response on both sides or a striking side difference in this test without a history of vestibular disorders

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ZUSAMMENFASSUNG

Zur Frage ob und wie sich der Pneumatisationsgrad des Felsenbeines auf die kalorische Nystagmusreaktion auswirkt untersuchten wi 15 Probanden mit geringer Pneumatisation (P P) und 15 mit ausgedehnter (L P) Pneumatisation Nach einer Wasserspülung (44° u 30°C 30 cm³ 30 sec) wurde die kalorische Antwort mit ENG aufgezeichnet und nach den Parametern Max Geschwindigkeit der langsamen Phase max. Kulminationsfrequenz, Latenz, Dauer Zeitpunkt der max Reaktion ausgewertet Im statistischen Gruppenvergleich und im intraindividuellen Vergleich der nur einseitig wenig pneumatisierten Probanden fanden sich außer für die Dauer hochsignifikante Unterschiede ($p<0\,001$) Vergleicht man diese Ergebnisse mit einem frühe entwickelten Modell des kalorischen Nystagmus (Bock & Broman 1977) so ergibt sich eine gute Übereinstimmung für die P P -Gruppe

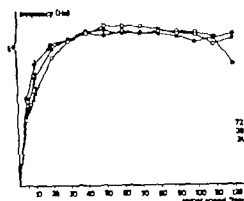


Fig 1 The correlation between the frequency of the OKN and the target speed The standard error of the mean is indicated at 10°/sec (O—O) rotator with 72 targets (●—●) rotator with 36 targets, (□—□) rotator with 24 targets

only minor significance has been attached to the target frequency Very often this is not stated in the text and it may even be impossible to calculate from the information given.

The aim of this paper is to emphasize the significance of the angular speed of the target and of the frequency of the targets for the regularity of the optokinetic response in normal human beings A standard procedure of the optokinetic test for clinical purposes is proposed

MATERIAL AND METHOD

The investigation was carried out on 6 healthy persons aged 18–39 years The subjects had no history of neurological or vestibular disease including cerebral commotion None of the subjects had abnormal spontaneous or positional nystagmus All had normal vision with or without corrective lenses No subject had taken any drugs or alcohol in the preceding 48 hours

Clockwise and counter-clockwise horizontal OKN was elicited by a rotator which projected lightslits onto a semicircular screen covering 140 degrees of the circumference in the horizontal plane and 30 degrees in the vertical plane Vertical OKN was not studied

Three rotators with equally spaced lightslits were used. The number of slits were 24 36 and 72 respectively Each subject was investigated three times with each of the rotators The target was presented in steps from 5 10 20 30 120 degrees/sec clockwise and counterclockwise making a total of 1404 recordings of OKN

Binocular eye deflections were recorded using an Elema mingograph under a.c amplification with a time constant of 2.5 sec Electrodes were attached lateral to both outer canthi and to the forehead above the bridge of the nose

Calibration was performed with eye deflections of 10 degrees every 30 sec in order to counteract the alteration of the corneo-retinal potential difference as the nature of the test made it impossible to obtain total darkness

The frequency and the eye velocity of the slow phase (EV) were calculated from a 10 second period of even response If the demand for a period of even response could not be fulfilled the test was discarded The EV was calculated by the method advocated by Jongkees

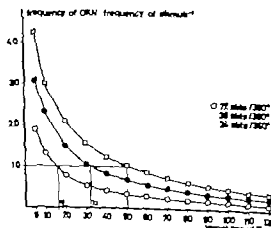


Fig 2 The ratio between the frequency of the OKN and the frequency of the targets correlated to the target speeds The ratio is 1/1 (synchronous response) at 16 °/sec (rotator with 72 targets O—O) 32°/sec (rotator with 36 targets, ●—●), and 50°/sec (rotator with 24 targets, □—□) The information in this figure gives the following general equation for synchronous response $A/360 \times B = 3.23$ where A is the number of targets per 360 degrees, and B is the target speed

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THE SIGNIFICANCE OF THE TARGET FREQUENCY AND THE TARGET SPEED IN OPTOKINETIC NYSTAGMUS (OKN)

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Abstract The significance of the frequency of the stimuli (target frequency) and the angular velocity of the stimuli (target speed) on horizontal optokinetic nystagmus (OKN) was investigated in 6 normal human subjects using target frequencies from ½ Hz to 24 Hz and target speeds from 5°/sec to 100°/sec Regularity and reproductiveness of the OKN were obtained only in test conditions where each transit of targets was followed by an optokinetic response either as one beat or as a sequence of beats This is called synchronous response and was found when the target frequency was below 3 Hz and the target speeds below 20–30°/sec depending on the actual frequency At higher target frequencies and target speeds the eyes were unable to take up every target resulting in uneven responses (hyposynchronous response) Very low target frequencies and target speeds were likewise unsuitable due to drifting of the eyes from one target to another A target frequency and a target speed close to the upper limit for synchronous response is advocated in clinical tests of OKN 2 Hz and 20°/sec is proposed as a suitable combination of frequency and target speed

Optokinetic nystagmus (OKN) may be defined as involuntary rhythmic movements of the eyes caused by unidirectional motion of the visual field

OKN is characterized by alternating slow and fast eye movements due to change in the fixation OKN is thereby distinguishable from pendular nystagmus which in normal persons is characterized by sinusoidal eye movements as the fixation of the eyes is constant

Attention is essential (ter Braak 1936) and OKN should never be tested while diverting the subject In our opinion the best way to secure a lasting arousal has been to ask the subject to count the moving stripes or targets Otherwise the subject tends to lose interest in the test situation which results in an uneven nystagmus unfit for scientific or clinical purposes

Since the classical works of Bárány (1921) and Cords (1926) several authors have stressed the fact that OKN is a reliable test to disclose lesions especially in the temporal lobe of the cerebrum but also in the cerebellum and the brain stem and that OKN should be included in the test battery of every thorough otoneurological examination Nevertheless the test has never been standardized for clinical purposes although several suggestions have been made (Blomberg 1960 Suzuki & Komatsuzaki 1962 Mizukoshi et al 1977) Furthermore the literature is to some extent contradictory concerning optimum test conditions

The two variables of major significance to the regularity of the OKN are the target speed and the target frequency The maximum angular velocity of a moving stimulus or target which may be accurately followed by the human eye is about 30 /sec When the velocity of the target exceeds this value the eye movements lag behind and are frequently interspersed with saccadic movements which tend to reduce the constantly accumulating errors of positioning (Westheimer 1954) In optokinetic tests substantially faster target speeds involve large variations of the velocity in the slow phase (Miyoshi & Pfaltz, 1974 Mizukoshi et al 1977) The maximum frequency at which the eye may fixate on every target of the optokinetic drum is 3–4 Hz in normal human subjects (Ino 1970) In the literature



Fig. 4. Optokinetic response to slow moving targets. The slow phases are interrupted by saccades in both directions due to poorly sustained fixation upon the targets. The theoretical response with preservation of fixation on every target is indicated by the dotted line.

The distribution of the coefficients of variation (CV) i.e. the ratios of the standard deviation to the mean value ($s/\bar{x}$) is shown in Fig. The CV is high at low speeds 10°/sec or less. For the faster speeds no systematic changes in the CV are noticeable. The reason for the high level at low speeds seems to be one of two possibilities. (1) at very slow speeds the eyes tend to drift from one target to another both in the direction of the movement and in the opposite direction thus breaking the uniformity of the nystagmus (Fig. 4). (2) One transit of a target before the eyes may result in a single nystagmic beat (primary beat) a larger nystagmic beat plus one or more small beats (secondary beats) or the nystagmus may be composed entirely of several small beats with no visible connection between the stimulus and the optokinetic response (secondary beats) (Fig. 5).

Eye velocity of the slow phase (EV)

The mean EV increased with the increased target speed from 5°/sec to 80–90°/sec (Fig. 6). A further rise in the target speed resulted in a slight decrease of the EV. The gain of the nystagmus i.e. the quotient EV×target speed^{-1} exceeds 100% for the lower speeds where the frequency of the OKN exceeds the frequency of the targets (hypersynchronous response). The gain of the OKN was 100% at about 30°/sec and less than 100% beyond this in the hypersynchronous area. This is in agreement with Westheimer's (1954) observations of the gain of smooth pursuit movements of the human eye.

There was no systematic difference in the area of 100% gain between tests with the three different rotators. This indicates that the gain of the nystagmus is independent of the target frequency.

The variation coefficient of the EV indicated two levels (Fig. 3): (1) a low level for target speeds close to and below the area of synchronous response and (2) a high level for the target speeds in the hyposynchronous area which coincided with the occurrence of uneven responses which were discarded due to the demand for regularity.

DISCUSSION

Generally it is recommended to investigate OKN at that particular target speed which secures a maximum response judged by the EV, the frequency of the OKN or the sum of the amplitudes (Enoksson 1956, Bergmann et

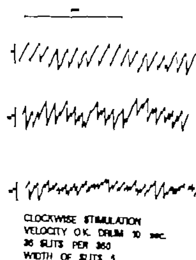


Fig. 5. Three patterns of optokinetic response. *Top row.* The amplitudes are of equal height. Each nystagmic beat represents the transit of one target (primary beat). *Middle row.* The OKN is composed of larger beats (primary beats) and small beats which do not correspond to the transit of the targets (secondary beats). *Bottom row.* The OKN is almost entirely composed of small, secondary beats.

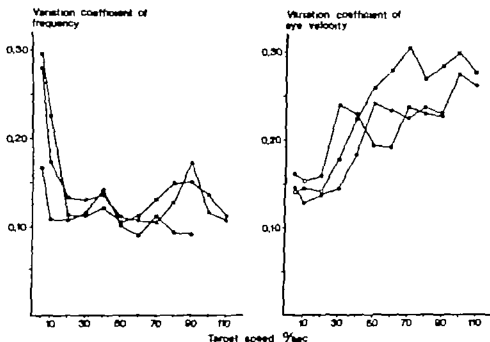


Fig 3 The correlation between the target speed and the coefficient of variation for the frequency of the OKN (*left*) and the coefficient of variation for the eye velocity of the slow phase (*right*) (O—O) rotator with 72 targets, (●—●) rotator with 36 targets, (□—□) rotator with 24 targets

& Philipzoon (1964) without consideration of the time used by the fast phase In fact this value is slightly less than the true eye velocity but the error is insignificant

RESULTS

An analysis of the variances revealed no inter subject differences All 6 subjects were able to perform the required period of even optokinetic response up to a target speed of 30°/sec However with higher target speeds an increasing number of tests were discarded due to the demand for regularity and at 120°/sec only 5 of the 36 tests were accepted

Frequency of the OKN

The frequency of the OKN increased with increasing target speed until a level of 3 2–3 4 Hz was reached at 40°/sec Target speeds beyond 40°/sec did not influence the frequency of the OKN apart from a slight decline in response frequency at the faster speeds close to 120°/sec (Fig 1) Before the maximum level of the response frequency was reached the mean frequency differed with the three different rotators The mean frequency was highest in the case of the rotator with 72 lightslits and smallest with the 24-slit rotator The significance of this difference is indicated in the figure by the standard error of the mean at 10°/sec

The initial rapid increase in frequency of the OKN followed by a steady level indicated that no direct correlation exists between the frequency of the OKN and the frequency of the targets

The relationship between these two factors is shown in Fig 2 where the ratio between the frequency of the OKN and the frequency of the targets is given as a function of the target speeds At slower speeds the quotient is greater than 1 At target speeds of 50 32 and 16 /sec for the three rotators with 24 36 and 72 lightslits respectively the quotient is 1 and at faster target speeds the quotient is less than 1

From the *information* given in Fig. 3 it is possible to compute the target frequency most liable to secure an optokinetic response with equal frequency of the eye movements and the targets which may be called a synchronous response Accordingly response frequencies which exceed the target frequency may be called hypersynchronous responses and the reverse hyposynchronous response

of the targets should not exceed 30°/sec which is the upper limit for the eyes to follow a moving target with smooth eye movements The minor importance of this factor is obvious from other investigations where satisfactory results have been obtained at a faster target speed. Mizukoshi et al (1977) reported good results with a target speed of 60°/sec and a target frequency of 2 Hz but lower speeds were not investigated

The optokinetic response to a single target may be composed of one or several beats Brucher's definition of synchronous response as an identity between the frequency of the targets and the frequency of the responses seems unsatisfactory In our opinion synchronous response should be defined as optokinetic nystagmus when each transit of targets is followed by an optokinetic response either as one beat or as a sequence of beats This would make Brucher's term hypersynchronous response superfluous OKN may than be divided into two types. synchronous when each target is fixed and hyposynchronous when the frequency of the targets is too high for the eyes to follow so that one or several of the targets go unnoticed It is in this latter type of OKN that great variations occur which may even be regarded as pathological (false positives)

According to the revised definition of synchronous response a target frequency of 3 Hz is too high because it does not leave enough room for the occurrence of secondary beats This is confirmed by the present experiment where an increasing number of tests were discarded with increasing target frequency above 2 Hz due to the demand for regularity On the other hand the frequency should not be much lower than 2 Hz as low frequencies tend to produce variation in the frequency and the regularity of the eye movements as previously described (Fig 4) Thus 2 Hz seems to be a safe limit for an even optokinetic test. Also it must be expected that only test conditions close to the upper limits of synchronous responses may prove suitable to disclose a pathological optokinetic response This problem will be discussed in a future publication.

CONCLUSION

(1) The frequency of the targets (stimuli) plays an important role for the regularity and reproductiveness of the optokinetic response

(2) For clinical purposes the target frequency should not exceed the upper limit for synchronous response i e the eyes must be able to respond to each transit of targets

(3) The upper limit for synchronous response is inversely proportional to the target frequency and the target speed

ZUSAMMENFASSUNG

Die Bedeutung von der Frequenz und der Geschwindigkeit des optokinetischen Reizes der horizontalen OKN war bei 6 gesunden Versuchspersonen untersucht mit Veränderungen der Reizfrequenz zwischen 1/3 Hz und 24 Hz und Veränderungen der Reizstärke von 5°/sec auf 120°/sec. Der OKN war nur regelmäßig und reproduzierbar zu bezeichnen wenn die Augen jeden Streifen des Drehzylinders fixieren könnten, was als synchrone Reaktion bezeichnet wurde. Eine synchrone Reaktion wurde gefunden entweder als eine einzelne Nystagmuszuckung oder als mehrere Zuckungen für jeden Streifen, wenn die Reizfrequenz unter 3 Hz war und die Reizstärke zwischen 20° 30°/sec abhängig von der aktuellen Frequenz. Mit einer größeren Reizfrequenz und Reizstärke waren die Augen nicht imstande jeden Streifen zu fixieren und der OKN war unregelmäßig (hyposynchrone Reaktion) Sehr niedrige Reizfrequenzen und Reizstärken sind unvorteilhaft weil die Augen dann von einem Streifen zu dem anderen gleiten wollen Eine Reizfrequenz und eine Reizstärke gleich unter der oberen Grenze der synchronen Reaktion sei in klinischen Untersuchungen von OKN zu empfehlen Hz und 20°/sec ist als eine günstige Kombination der Reizfrequenz und der Reizstärke vorzuschlagen.

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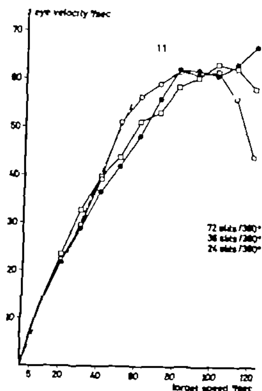


Fig 6 The correlation between the eye velocity of the slow phase and the target speed

al 1963 Honrubia et al 1968) The actual speed of the drum varies according to the equipment but is often between 60–90°/sec Mizukoshi et al (1977) in their acceleration test estimated the 60°/sec to be superior to the 90 /sec due to wider ranges in the normal response at the faster speed Several authors have observed large variations in the normal response at fast target speeds (Miyoshi & Pfaltz 1974) but even so the optokinetic fusion limit i e the upper limit for a continuous optokinetic response has been proposed as a measure of optokinetic normality (Blomberg 1960) On the other hand Honrubia et al (1968) have warned against too low target speeds due to large ranges of the frequency and of the amplitudes similar to the findings in this study However target speeds as low as 3 /sec have been used without any apparent problems (Coats 1968) Other authors have recommended the use of several different target speeds (as many as three) and have suggested the presence of a pathological response if asymmetry is obvious with one or more of these (Coats 1968 Enoksson 19 One of the reasons for the discrepancies in t advocated target speed may be the differ of the target frequency This factor is to som extent overlooked in the discussion of the o timum conditions for the OKN Miyoshi & Pfaltz (1973) claimed that the EV decreased accordance with the increase in the (frequency) of stimuli (targets) while the fre quency of the OKN increased with an increa ing number of targets up to a value of 1 360 degrees Other authors have found li or no influence on the frequency of the OK *of a change in the frequency of the targ* (Mackensen 1954 Enoksson 1956 Honrub et al 1967)

Brucher (1964) working with monkeys, *the first to stress the changing ratio betw* stimulus frequency and response frequen and introduced the terms *hypersynchrono synchronous* and *hyposynchronous* respons Smith & Bridgman (1943) measured the eff ciency of optic nystagmus" in the guinea p by an *index* based on the number of line passing a point in the visual field during eac response the nystagmus index In all of thei tests however the indices were greater than and the responses were in what Brucher re ferred to as the hyposynchronous area Hon rubia et al (1967) in the cat and Komatzusa et al (1969) in the monkey observed an ex cess of response frequency at low targe speeds but stressed the fact that the frequenc of the OKN was more dependent on the velo city of the optokinetic drum than on the num ber of stripes

Our findings indicate that test conditions which secure a synchronous response are su perior to tests where the maximum strength of optokinetics is elicited The two important conditions are (1) the eyes must be able to take up every target and (2) the eyes must be *able to cope with the speed of the targets* This *last factor* is less important than the first The frequency of the targets should not exceed *approximately 3 Hz since this is the upper* limit of fixation on all targets Also the speed

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VESTIBULAR UNITARY RESPONSES TO VISUAL STIMULATION IN THE RABBIT

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(Received July 17 1978)

ict Vestibular nucleus (VN) neurons, identified by idal rotation bout the vertical axis were tested ar response to moving visual field, and to electric ation on the optic chiasm (OX) and super colliculus Incremental responses during the rotation were in-d by visual stimulus in cerebellectomized animals. :-pulse stimulation applied to the OX wa ineffective ducing positive response in these neurons as well any other unit Thus such visual pathway to VN be located inside the brainstem and contains multi-ses. Effects of repetitive stimulations (60 Hz) to OX SC were more predominant in the cerebellectomized d than in the intact animal. Cerebellar inhibitory ol on visual-vestibular linkage was suggested.

: integration of visual and vestibular inputs iost important to maintain proper coordina- ı of the eye head and body movements : vestibular nuclei (VN) are considered to one of the visual–vestibular integrative iters as both lesion placement in vestibular :lei and labyrinthectomy decrease the slow ase eye speed of optokinetic nystagmus and minate the optokinetic after nystagmus ohen et al. 1973 Uemura & Cohen 1973 llewijn 1976 Zee et al 1976)

It has been established recently by means microelectrode technique that VN neurons hich respond to rotatory stimulation also re- pond to optokinetic stimulation in the gold- ih (Allum et al 1976) rabbit (Dichgans & randt 1972) and rhesus monkey (Waespe & 'enn, 1977) However little is known about e linkage from the visual system to the VN nd related synaptic events

The experiment reported here attempted to lemonstrate how VN neurons, some of which have been influenced by moving visual field stimulation respond to optic nerve and superior colliculus stimulations

METHODS

Twelve albino rabbits 8 intact and 4 cerebellectomized were used in this experiment These were anesthetized with urethane (1.2 g/ kg, i p) The trachea and femoral vein were cannulated and immobilized with gallamine triethiodide (8–10 mg/kg, i v) and the animals were maintained on artificial respiration

The animal's head was fixed on a stereotaxic frame The inclination was such that the bregma was 1.5 mm above the level of lambda (Sawyer et al 1954) Thus, the vertical canals as well as the horizontal canals could be stimulated by table rotation about the vertical axis (i e sinusoidal rotation controlled by a d c. motor) The table was rotated in an earth-fixed white screen with vertical black stripes either in the dark or in the light. Thus the rotation in the dark resulted in pure vestibular stimulation and that in the light resulted in visual and vestibular stimulation combined

Bipolar steel electrodes were inserted into the optic chiasm (OX) and superior colliculus ipsi- and contralateral to the recording site (SCi and SCc) Final electrode tip position was assessed by using the mass responses to a

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Abstract. Vestibular nucleus (VN) neurons, identified by sinusoidal rotation about the vertical axis, were tested for their response to moving visual field, and to electric stimulation on the optic chiasm (OX) and superior colliculus (SC). Incremental responses during the rotation were inhibited by visual stimulus in cerebellectomized animals. Single-pulse stimulation applied to the OX was ineffective in producing positive response in these neurons as well as in any other unit. Thus, such a visual pathway to VN must be located inside the brainstem and contains multi-synapses. Effects of repetitive stimulations (60 Hz) to OX and SC were more predominant in the cerebellectomized animal than in the intact animal. Cerebellar inhibitory control on visual–vestibular linkage was suggested

The integration of visual and vestibular inputs is most important to maintain proper coordination of the eye, head and body movements. The vestibular nuclei (VN) are considered to be one of the visual–vestibular integrative centers, as both lesion placement in vestibular nuclei and labyrinthectomy decrease the slow phase eye speed of optokinetic nystagmus and eliminate the optokinetic after-nystagmus (Cohen et al. 1973, Uemura & Cohen 1973, Collewijn 1976, Zee et al. 1976).

It has been established recently, by means of microelectrode technique, that VN neurons which respond to rotatory stimulation also respond to optokinetic stimulation in the goldfish (Allum et al. 1976), rabbit (Dichgans & Brandt 1972) and rhesus monkey (Waespe & Henn 1977). However, little is known about the linkage from the visual system to the VN and related synaptic events.

The experiment reported here attempted to ... some of which have been influenced by moving visual field stimulation, respond to optic nerve and superior colliculus stimulations.

METHODS

Twelve albino rabbits, 8 intact and 4 cerebellectomized, were used in this experiment. These were anesthetized with urethane (1.2 g/kg, i.p.). The trachea and femoral vein were cannulated and immobilized with gallamine triethiodide (8–10 mg/kg, i.v.) and the animals were maintained on artificial respiration.

The animal's head was fixed on a stereotaxic frame. The inclination was such that the bregma was 1.5 mm above the level of lambda (Sawyer et al. 1954). Thus the vertical canals as well as the horizontal canals could be stimulated by table rotation about the vertical axis (i.e. sinusoidal rotation controlled by a d.c. motor). The table was rotated in an earth-fixed white screen with vertical black stripes either in the dark or in the light. Thus the rotation in the dark resulted in pure vestibular stimulation and that in the light resulted in visual and vestibular stimulation combined.

Bipolar steel electrodes were inserted into the optic chiasm (OX) and superior colliculus ipsi- and contralateral to the recording site (SCi and SCc). Final electrode tip position was assessed by using the mass responses to a

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STIMULATING SITES

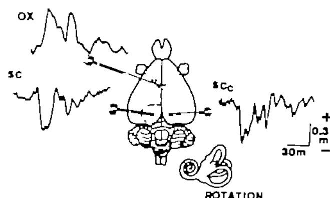


Fig 1 The figure shows stimulus sites in the visual system and optically evoked responses recorded in these areas The electrode tip on OX was usually fixed about 1 mm above the skull base thus ensuring the amplitude of evoked response was large enough The electrode tip position in SC was about .5–3.5 mm caudal to the rostral edge of SC 1– mm lateral to the midline and 2.5–3.5 mm deep from the surface of SC. Recording was done from left VN

flash of light (Fig 1) Electrical pulses of 0 05 msec and varying intensity were applied repetitively at a rate of 50–70 Hz for 5–10 sec

Vestibular neuronal activity was extracellularly recorded by a glass microelectrode filled with lithium carmine or 3 M KCl Representative recording sites were labeled by dye deposition (Mitarai 1960) Unitary activity was displayed on an oscilloscope using a conventional circuit Discharge frequency was recorded on oscillographic paper through the pulse discriminator and F V converter

At the end of the experiment each brain was perfused with 10% formalin saline and embedded in paraffin Recording sites were verofoed om 30 μm serial coronal sections stained with cresyl violet

RESULT

A total of 71 VN neurons responding to rotational stimulation were tested by OX stimulation These were mostly located in medial VN (65 units) and others were in superior VN (3 units) and in lateral VN (3 units) Fig 2 A shows the discharge frequency of a type II VN neuron which is excited by contralateral rotation and inhibited by ipsilateral rota (Duensing & Schaefer 1958) of a cerebellectomized animal during rotation in the d and in light The excitatory responses by vestibular stimulation (clockwise rotation are partly inhibited by the presence of vis signal Such differences in response found in 7 out of 22 units in cerebellectom animals however 7 neurons (including 2 p tive units) were found by histological exami tion to be located in the abducens nucleu and peri VI nucleus

Fig 2B shows the firing rate of the neuron shown in Fig 2A during repetiti stimulation of OX ipsi and contralateral SC A weak intensity stimulus of OX inhibits the discharge (*a*) in contrast a strong stimulu induces facilitation and nystagmic bursts (*b*) On visual inspection we ascertained th these bursts coincided with left-beating ocula nystagmus Ipsi- and contralateral SC stimula tions cause similar facilitatory responses (*c* and *d*)

Effects of repetitive stimulation of OX upon vestibular unitary activity are summarized in Table I As the response sensitivity of each unit was variable each column included a wide range of changes in discharge frequency Inhibition of the firing was generally converted into facilitation when stimulus intensity was increased (Fig 2B *a* and *b*) As a result there were few units showing an inhibitory response The intact animal group contains more no response changes" than did the cerebellectomized group Such a difference in responsiveness between the intact and cerebellectomized animal groups was significant (χ^2 test $p<0\ 01$) Between type I and II neuron groups the frequency ratios of negative and positive responses did not differ significantly (not shown in Table)

In 32 neurons the responsiveness to OX SCi and SCc stimulations could be examined successively (Table II) Seven units did not respond to stimulation of three different sites while 15 units exhibited a uniform response

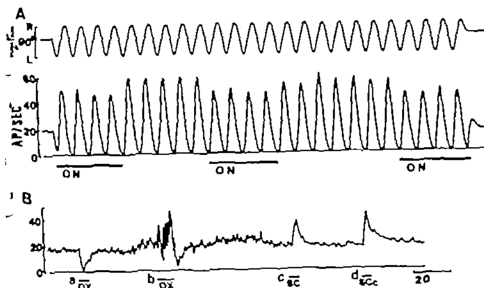


Fig. 2. Discharge rate of type-II neuron. The neuron was located in the lateral border of left medial VN. Its firing increased during clockwise rotation and decreased during counterclockwise. Straight lines (marked with *ON*) under the discharge curve in (A) indicate the presence of light. Note that incremental responses with the light ON are consistently smaller than those in the dark. (B) Discharge rate of same neuron shown in (A). I ( ) OX is repetitively stimulated at 60 Hz by small electric current (2 V) and in (*b*) by larger current (9 V). Discharge is inhibited in ( ) and increased in (*b*). Lines ( ) and (*d*) indicate repetitive stimulations applied on ipsi- and contralateral SC, respectively. Similar excitatory responses are shown.

(i.e. either facilitation or inhibition). Varying combinations of responses were observed in the remaining 10 units.

DISCUSSION

Repetitive stimulation applied to OX or SC occasionally induced ocular nystagmus when the effect of the drug (gallamine triethiodide, a neuromuscular transmission inhibitor) waned. In these cases some VN unit activity was found to be coincidental with the ocular movement. This result is consistent with observations in squirrel monkeys which showed that vestibular neuronal excitation is maintained as long as the ocular nystagmus continues (Waespe & Henn 1977*a*, 1977*b*).

Recordings from the VN of several species (Dichgans & Brandt 1972, Allum et al. 1976, Waespe & Henn 1977) indicated some nonlinearities between optokinetic stimulus and vestibular response such as considerable time lag and saturation effect. It is noted in these

Table I. *The numbers and percentages of VN neurons examined by repetitive stimulation on OX*

	No response	Facilitation	Inhibition	Total
Intact	27 64.3 %	11 26.2 %	4 9.5 %	42 100 %
Cerebellectomized	6 20.7 %	19 65.5 %	4 13.8 %	29 100 %

Table II. *Response characteristics of VN neurons to OX, SCi and SCc stimulations*

Number of unit	No response	Facilitation	Inhibition	Others
32	7	12	3	10

reports that there was a less direct connection between the visual system and the VN than between the vestibular labyrinth and the VN

Since the effects of repetitive stimulations on SC were usually no stronger than that of OX stimulation it is unlikely that the SC lies in the midway of the path from the optic nerve to the VN Hobbelen & Collewijn (1971) and Collewijn (1975) also suggested that the pretectal nucleus is a more likely relay station for optokinetic signals to the oculomotor nuclei than the superior colliculus It was shown by Maekawa & Simpson (1973) that visual impulses through the accessory optic tract and pretectum were transmitted to the cerebellar flocculus via the inferior olive Therefore this pretecto-olivo-cerebellar pathway has been considered to be one of the responsible visual paths transmitting optokinetic signals to the VN (Allum et al 1976 Waespe & Henn 1977) It is obvious in this experiment however that visual signals influence VN unit activity even in the absence of cerebellum Therefore it may be postulated that another visual pathway to the VN exists inside the brainstem Since in our previous paper (Kubo et al 1978) the pontine reticular neurons were described as responding to single shock stimulation of OX with relatively short latency such visual cells in the brain stem may transmit optokinetic information to the VN Present results VN neurons do not respond to single shock stimulus applied to OX but do respond to repetitive stimulation thus indicating multisynaptic connections between the visual and vestibular systems

The cerebellum is considered to play a role in modifying visual–vestibular interaction In cerebellectomized animals unitary discharges during rotation were influenced by the presence of a visual stimulus whereas no positive unit was found in the intact animals (19 units were examined) The response of the VN unit to OX stimulation was no more frequently observed in the intact animal than in the cerebellectomized These differences may reflect the cerebellar inhibitory control of the vestibular system (Highstein 1973 Robinson 1976) However since alertness of the animal is a very important factor when inducing optokinetic response in the VN (Waespe & Henn 1977*a*) and the number of units is limited, the present experimental findings do not necessarily indicate complete obliteration of the visual signal by the cerebellum in the intact animal

ADDENDUM

After this paper was accepted the report that demonstrated positive effect of optokinetic stimulation on vestibular nucleus neurons of cerebellectomized cats was published (Keller E L & Precht W 1978) Persistence of visual response in vestibular nucleus neurons in cerebellectomized cat *Exp Brain Res* 32 591

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ZUSAMMENFASUNG

Vestibuläre Nukleus-Neurone (VN) die sich durch eine höhlenförmige Rotation um die vertikale Axis kennzeichnen wurden auf ihre Reaktion zu einem in Bewegung stehenden Sichtfeld und zu einer elektrischen Anregung auf die Sehnervenkreuzung (OX) und Super-Colliculus (SC) geprüft Die zunehmenden Reaktionen während der Rotation wurden durch eine visuelle Reizung in zerebellierten Tieren gehemmt Eine einzelne Pulsanreizung auf die OX war ohne Wirkung eine positive Reaktion in diesen Neuronen hervorzurufen. Desgleichen auch in allen anderen Einheiten. Infolgedessen muß solch eine Sehnervenbahn zu den VN im inneren des Hirnstammes gelegen sein und viele Nervenübergangsstellen haben Die Resultate von wiederholten Anreizungen (60 Hz) auf die OX und SC ware mehr überwiegend in den zerebellierten Tieren als in normalen Tieren Eine zerebellare, hemmende Kontrolle auf die visuelle vestibulare Verbindung wurde vorgeschlagen

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reports that there was a less direct connection between the visual system and the VN than between the vestibular labyrinth and the VN

Since the effects of repetitive stimulations on SC were usually no stronger than that of OX stimulation it is unlikely that the SC lies in the midway of the path from the optic nerve to the VN Hobbelen & Collewijn (1971) and Collewijn (1975) also suggested that the pretectal nucleus is a more likely relay station for optokinetic signals to the oculomotor nuclei than the superior colliculus It was shown by Maekawa & Simpson (1973) that visual impulses through the accessory optic tract and pretectum were transmitted to the cerebellar flocculus via the inferior olive Therefore this pretecto-olivo-cerebellar pathway has been considered to be one of the responsible visual paths transmitting optokinetic signals to the VN (Allum et al 1976 Waespe & Henn 1977) It is obvious in this experiment however that visual signals influence VN unit activity even in the absence of cerebellum Therefore it may be postulated that another visual pathway to the VN exists inside the brainstem Since in our previous paper (Kubo et al 1978) the pontine reticular neurons were described as responding to single shock stimulation of OX with relatively short latency such visual cells in the brainstem may transmit optokinetic information to the VN Present results VN neurons do not respond to single shock stimulus applied to OX but do respond to repetitive stimulation thus indicating multisynaptic connections between the visual and vestibular systems

The cerebellum is considered to play a role in modifying visual–vestibular interaction In cerebellectomized animals unitary discharges during rotation were influenced by the presence of a visual stimulus whereas no positive unit was found in the intact animals (19 units were examined) The response of the VN unit to OX stimulation was no more frequently observed in the intact animal than in the cerebellectomized These differences may reflect the cerebellar inhibitory control of the vestibular system (Highstein 1973 Robinson, 1976) However since alertness of the animal is a very important factor when inducing optokinetic response in the VN (Waespe & Henn, 1977*a*) and the number of units is limited the present experimental findings do not necessarily indicate complete obliteration of the visual signal by the cerebellum in the intact animal

ADDENDUM

After this paper was accepted the report that demonstrated positive effect of optokinetic stimulation on vestibular nucleus neurons of cerebellectomized cats was published. (Keller E L. & Precht W 1978) Persistence of visual response in vestibular nucleus neurons in cerebellectomized cat *Exp Brain Res* 32 591

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ZUSAMMENFASUNG

Vestibulare Nukleus-Neurone (VN) die sich durch eine höhlenförmige Rotation um die vertikale Axis kennzeich-nen wurden auf ihre Reaktion zu einem in Bewegung stehenden Sichtfeld und zu einer elektrischen Anregung auf die Sehnervenkreuzung (OX) und Super-Collicul (SC) geprüft Die zunehmenden Reaktionen während der Rotation wurden durch eine visuelle Reizung in zere-bellierten Tieren gehemmt. Eine einzelne Pulsanregung auf die OX war ohne Wirkung, eine positive Reaktion in diesen Neuronen hervorzurufen Desgleichen auch in allen anderen Einheiten. Infolgedessen muß solch eine Sehnervenbahn zu den VN im inneren des Hirnstamme gelegen sein und viele Nervenübergangsstellen haben Die Resultate von wiederholten Anregungen (60 Hz) an die OX und SC wäre mehr überwiegend in den zerebel-lierten Tieren als in normalen Tieren Eine zerebellare hemmende Kontrolle auf die visuelle vestibulare Verbin-dung wurde vorgeschlagen

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Table I *Influence of myringotomy on recovery from acute otitis media*

	Recovered (%)	
	Myringotomy (N=68)	No myringotomy (N=90)
Follow-up		
After 2 weeks	71	42 $P<0.001$
After 4 week	90	71 $P<0.01$

cotton swab attached to a metallic thread Myringotomy was not performed on the next 90 children. Specimens for bacterial culture from these children were taken from the nasopharynx as in the previous group These children received pain-relieving ear drops (Chloprin c anaesthetico®) which the parents had permission to apply temporarily while the child complained of earache In addition the parents were advised to come again to the Ear Department if the child's earache did not subside or if fever persisted

All the children received 80000–100000 IU/kg/24 hrs of V penicillin p o divided into three doses for a 10-day period

In both groups the follow-up examinations were performed by the same doctor who had performed the initial clinical examination The first follow-up examination was performed 14 days and the second 28 days from the occurrence of the initial infection The middle ear infection was regarded as having been cured/not cured on the basis of the same criteria upon which the initial diagnosis had been made Myringotomy was not performed at the first follow-up examination even though the infection persisted if it had not initially been performed In the group that had initially been treated with myringotomy remyringotomy was performed if necessary at both follow-up exams In the group in which myringotomy had not been performed initially myringotomy was performed only at second follow-up exam if necessary

If the middle-ear infection persisted at the time of the first follow-up examination the children received p o amoxicillin 20 mg/kg/24 hours divided into three doses for a 10-day period

Bacterial culture specimens were taken in conjunction with both follow-up exams both from the middle ear and from the nasopharynx as at the initial examination All specimens were cultured immediately onto plates

RESULTS

Of the children treated initially with myringotomy 71% (48/68) were cured at the time of the first follow-up and 90% were cured at the second follow-up Of the children treated initially without myringotomy 42% (38/90) were cured at the time of the first follow-up and 71% (64/90) at the time of second follow-up (Table I) Myringotomy had to be performed on 5 of the children from the latter group before the first follow-up due to either persisting fever or ear pain. In addition myringotomy had to be performed on one child from the same group between the two follow-ups even though he had been judged as having been cured at the time of the first follow-up

Amoxicillin had to be given to 29% (20/68) of the myringotomy patients at the first follow up and to 49% (44/90) of the patients in the no-myringotomy group

At the second follow-up myringotomy had to be performed on 10% (7/68) of the patients initially treated with myringotomy and on 22% (20/90) of those initially treated without myringotomy

81% (25/31) of the "severe" middle-ear infections had been cured at the time of the second follow-up exam in the myringotomy group and correspondingly 57% (20/35) in the group treated without myringotomy

If the patient had not previously had any middle-ear infections the cure rate was 100% (16/16) in the myringotomy group compared to 87% (26/30) in the group treated without myringotomy If the children had previously had 1–2 middle-ear infections the corresponding figures were 100% (20/20) in the myringo-

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MYRINGOTOMY IN THE TREATMENT OF ACUTE OTITIS MEDIA IN CHILDREN

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Abstract The treatment of acute otitis media was studied in 158 children All children (mean age 4 years) received penicillin orally 80000–100000 IU per day for 10 days Myringotomy was performed on 68 children at the time of the diagnosis The other 90 children were treated with penicillin and ear drops The bacteriological findings from the nasopharynx at the time of diagnosis were equivalent in both groups After weeks 42% of the children without myringotomy and 71% of the children with myringotomy were cured The children who were not cured were treated with amoxicillin for 10 days Four weeks after diagnosis 71% and 90% of the children respectively were cured The differences between the two groups are significant The observations indicate that myringotomy clearly accelerates the recovery from acute otitis media

Acute otitis media is a common complication of feverish colds in small children The correct diagnosis is more difficult to make than is commonly realized Ear pain may be absent in more than 75% of cases (Bluestone & Shurin 1974) The treatment of otitis media today consists according to present practice of antibiotics and myringotomy (Palva & Karma 1973) The necessity of myringotomy however has been debated Failure to perform myringotomy is claimed by some to increase the risk of developing secretory otitis media On the other hand otitis media has been shown to be cured with the use of antibiotics alone (Roddey et al 1966 Herberts et al 1971 Bergholtz & Rudberg 1972 Lorentzen & Haugsten 1977) In this study the effect of myringotomy on the recovery from acute otitis media in children has been examined

PATIENTS AND METHODS

The material for this study consisted of 158 children who had come during calling hours to the Ear Department of Turku University Central Hospital (TYKS) because of ear pain or suspected acute otitis media. The majority of the children were below school age the youngest was 6 months and the oldest 15 years. The average age was 4 years 88 of the patients were girls and 70 were boys

The parents were questioned as to the number of previous middle-ear infections and their treatment Prerequisites for inclusion in the study were that antibiotic therapy had not previously been instituted for the present infection and that the child was not allergic to penicillin

The diagnosis of acute otitis media was made on the basis of the redness bulging and mobility of the tympanic membrane These findings were subjectively graded on a scale of 0 1 and 2 0 signified normal and 2 a clearly pathologic finding When all three parameters were graded as 2 the infection was regarded as being severe"

The patients were divided for the study into two groups Myringotomy and complete removal of the inflammatory secretions by suction were performed on the first 68 children. Specimens for bacterial culture were taken from both the middle-ear secretions and transnasally from the nasopharynx with a charcoal

secretions in the middle-ear cavity. Such authors would like to limit the use of myringotomy to cases in which pain cannot be controlled by other means (Rowe 1975). It is apparent however that failure to perform myringotomy fosters the development of secretory otitis media. The present study clearly shows that myringotomy is necessary in the treatment of acute otitis media in children despite improvement in the antibiotics available. On the other hand mild middle-ear infections can be treated with antibiotics alone but it is necessary in these cases too to insure that a follow-up exam is performed possibly including a hearing examination. Myringotomy should always be performed in cases of recurrent otitis media.

Penicillin in doses of 80 000–100 000 IU/kg/24 hrs p.o. which is effective against the majority of haemophilus bacteria has been recommended in the primary treatment of acute otitis media (Lundgren 1972). However it is worth mentioning that G-penicillin has been shown to be more effective than V-penicillin against haemophilus (British Medical Journal 1976). In this study 71% of the children in the myringotomy group recovered with penicillin. Amoxicillin had to be employed with 29% of the myringotomy patients, and with 49% of those treated initially without myringotomy. The choice of antibiotic in very young children can be more difficult because the incidence of haemophilus infections in younger children is greater than in older children. For this reason ampicillin or amoxicillin has been recommended as primary treatment (Laxdal et al. 1970). Erythromycin–sulfa combinations have been considered to be almost equally effective alternatives (Howie & Ploussard 1972, Howard et al. 1976).

The results of bacterial cultures from the nasopharynx are known to correlate well with those of the middle ear (Lundgren 1972, Nylén 1975). In this study the most common bacteria in the nasopharynx were pneumococcus and haemophilus, and can be regarded as the cause of the otitis media. Worth noting is the fact that the incidence of haemophilus in the nasopharynx did not markedly decrease after either penicillin or amoxicillin therapy. In contrast the incidence of penumococcus in the nasopharynx cleary decreased as a result of penicillin therapy. Haemophilus was cultured from the nasopharynx in 48–55% of children who had not recovered. Haemophilus was also surprisingly abundant in the cultures of the nasopharynx of those children who had recovered. It seems that despite antibiotic treatment, haemophilus appears abundantly in the nasopharynx and this at least partially explains recurrent otitis media.

Recurrent otitis media in children can create a real problem in therapy. The increase in the number of children in various forms of daycare at least partially explains these continually recurring infections. Children who are cared for at home have been shown to have fewer respiratory and middle-ear infections than day care centre children (Strangert, 1976).

The role of allergy in the development and recurrence of otitis media in children is obvious. 15% of children under school age are atopic and they are known to have more frequent middle-ear infections than healthy children (Kjellman 1977). The role of food allergy as a cause of otitis media has also been discussed (Wilson 1971). At least in the treatment of children with secretory otitis media an attempt should be made to clarify an allergic etiology and to take this possibility into account in planning treatment (Viscomi 1975).

ZUSAMMENFASSUNG

158 Kinder mit akuter Mittelohrentzündung wurden untersucht. Alle Kinder (durchschnittlich vier Jahre alt) erhielten 80 000–100 000 I.E. Penicillin per os täglich während zehn Tagen. Trommelfellpunktionen wurden bei 68 Kindern gemacht. Die anderen 90 Kinder wurden mit Penicillin und Ohrentropfen behandelt. Die Bakterien von Nasopharynx waren in beiden Gruppen ähnlich. Nach zwei Wochen waren 42% von Kindern ohne Punktion und 71% von Kindern mit Punktion geheilt. Die Kinder, die nicht geheilt waren, erhielten dann Amoxicillin während zehn Tagen. Nach vier Wochen von Diagnosis waren 71% und 90% der Kinder geheilt. Die Differenzen zwischen

Table II *Recovery from acute otitis media in myringotomy and no-myringotomy groups 4 weeks after initiation of treatment compared with the number of previous middle-ear infections*

Previous middle-ear infections	Recovery rate (%)
Myringotomy	
0	100 (16/16)
1–2	100 (20/20)
more than 2	78 (25/32)
No myringotomy	
0	87 (26/30)
1–2	62 (13/21)
more than 2	64 (25/39)

Table IV *Results of bacterial culture of the nasopharynx of those children who had not recovered from otitis media at the time of the first follow-up exam (%)*

All children had been treated with penicillin for 10 days

	Myringotomy (N=20)	No myringotomy (N=52)
Diplococcus pneumoniae	15	4
Haemophilus influenzae	55	48
Streptococcus pyogenes (A)	0	0
Staphylococcus aureus	10	
Non-pathogenic bacteria or negative culture	20	26

tomy group and 62% (13/21) in the no-myringotomy group On the other hand if the children had had three or more middle-ear infections the corresponding figures were 78% (25/32) and 64% (25/39) (Table II)

The results of the bacterial cultures taken transnasally from the nasopharynx at the initial examination are presented in Table III The most common bacteria were pneumococcus and haemophilus There were no significant differences between the two groups in regard to the results of the bacterial cultures Haemophilus was found in the culture of the nasopharynx taken at both follow-up exams in both groups with about the same frequency as before initiation of treatment In contrast pneumococcus was cultured clearly less frequently after treatment than before Haemophilus was the most common bacteria in the nasopharynx after penicillin therapy in those children in whom the middle-ear infection persisted at the time of the first follow-up (Table IV) In this respect there was no significant difference between the two groups At the time of the diagnosis haemophilus was cultured from the nasopharynx of 41 of the 158 children making up the entire material Haemophilus persisted in the nasopharynx in 33% of these, despite penicillin therapy 22 of these 33 children were classified as not having recovered at the time of the first follow-up and received amoxicillin after which haemophilus could no longer be cultured from the nasopharynx at the time of the second follow-up in 8 of these 22 children

Table III *Results of bacterial cultures taken from the nasopharynx in both the myringotomy and no-myringotomy groups before initiation of treatment (%)*

	Myringotomy (N=68)	No myringotomy (N=90)
Diplococcus pneumoniae	31	34
Haemophilus influenzae	30	26
Diplococcus pneumoniae + Haemophilus influenzae	6	1
Streptococcus pyogenes (A)	6	3
Staphylococcus aureus	3	1
Non-pathogenic bacteria or negative culture	4	35

DISCUSSION

Numerous studies have been presented in the literature in which there is an attempt to minimize the importance of myringotomy in the treatment of otitis media in children (Roddey et al 1966 Herberts et al 1971 Bergholtz & Rudberg 1972 Lorentzen & Haugsten 1977) It has even been suggested that myringotomy acts as trauma which stimula

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NON TRAUMATIC CEREBROSPINAL RHINORRHEA AND CHONDRODYSTROPHY

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(Received May 29 1978)

Abstract Two chondrodystrophic males have developed non-traumatic cerebrospinal rhinorrhea within their second decade Hydrocephalus was the cause of cerebrospinal fluid leakage in both cases. To lower the intracranial pressure the neurosurgeon performed ventriculocardiostomy The fistula was closed by the rhinosurgical extradural route With respect to the atrophic brain in cases of hydrocephalus, the fronto-nasal approach appeared to be the best and less traumatic way of exposing the frontal base of the skull

The clinical entity of cerebrospinal rhinorrhea was described by Sir St Clair Thomson in 1899 He selected 21 cases of so-called spontaneous cerebrospinal fluid fistulas" from the literature As the term spontaneous cerebrospinal fluid rhinorrhea" is considered not to be very exact many authors prefer a classification of traumatic and non-traumatic" rhinorrhea (Table I) Traumatic cerebrospinal rhinorrhea is a relatively common event for neurosurgeons and ENT specialists while the non-traumatic form is seldom observed

In this report we describe patients with non-traumatic cerebrospinal rhinorrhea and chondrodystrophy Hitherto this combination has not been described

CASE REPORTS

A male aged twenty was first seen at the age of seventeen. Except for chondrodystrophy he had an uneventful history up to his fifteenth year when a watery clear fluid was dripping from his left nostril when bending his head forward. On admission we considered hydrocephalus The hydrocephalus was confirmed by X-ray of the skull encephalography after insufflation of air and ventriculography Furthermore a pneumatocephalus behind the right frontal sinus was visible As shown in the X ray plate (Fig. 1) the frontal brain was extremely atrophied A fluid level in the left maxillary sinus (Fig 2) could be verified to be cerebrospinal fluid The site of the cerebrospinal fluid leakage could not be detected but according to the clinical signs it was thought to be on the left side

As the hydrocephalus was due to obstruction of the cerebrospinal fluid circulation in the posterior cranial fossa a neurosurgical decompression of the posterior fossa was done and an Arnold-Chiari malformation was discovered As this intervention failed to lower the intracranial pressure a cardioventriculostomy was performed The cerebrospinal rhinorrhea ceased but the areocele did not resorb Surgery by the fronto-nasal approach on the left frontal base of the skull was unsuccessful in detecting the cerebrospinal fluid fistula Because of recurrence of cerebrospinal rhinorrhea and pneumatocephalus surgery by the fronto-nasal approach was done on the right side A round fistula 4 mm in diameter in the posterior wall of the frontal sinus was plugged with muscle fascial graft and tissue adhesive The patient has now been free of

den Gruppen waren statistisch bedeutend Die Trommelfellpunktion beschleunigte die Heilung der akuten *Mittelohrenentzündung*

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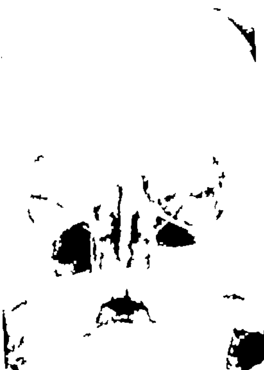


Fig. 2. Patient D. S. with cerebrospinal fluid level in the left maxillary sinus.



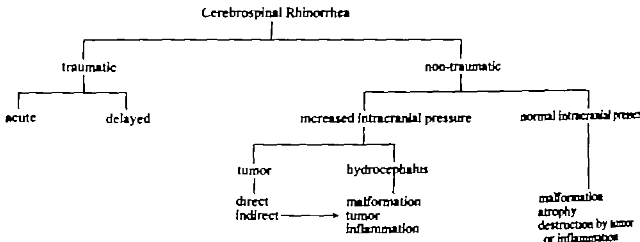
Fig. 3. Chondrodystrophic patient L. B. with hydrocephalus, spontaneous pneumatocephalus, enlargement of the lateral ventricle, brain atrophy and air bubble behind the frontal sinus.

hospital again. X-ray and tomography of the skull (Fig. 3) showed a spontaneous pneumatocele of both lateral ventricles, air in the subarachnoidal spaces and an air bubble behind the right frontal sinus. The brain was extremely atrophied. Surgery by the fronto-nasal approach on the right frontal base of the skull failed to detect a fistula, and the left side was therefore exposed again. A round fistula 3 mm in diameter in the most frontal area of the cribriform plate was plugged with fascial graft, cartilage and a mucosal flap fixed with tissue adhesive. No cerebrospinal rhinorrhea has been observed for more than 3 years. In the meantime the patient has developed a symptomatic epilepsy.

COMMENTS

Both of our patients with chondrodystrophy developed a manifest hydrocephalus and non-traumatic cerebrospinal rhinorrhea during their second decade. In both cases the hydrocephalus was unknown on admission and in one patient we detected the increased intracranial pressure only after surgical exposure of the frontal base of the skull. The cause of hydrocephalus was an Arnold-Chiari malformation in one case, in the other patient the obstruction of the subarachnoidal spaces could not be distinguished. In spite of scintigraphy, tomography and encephalography after insufflation of air, the site of the cerebrospinal fluid fistula could not be detected. Therefore both sides of the frontal base of the skull had to be exposed by the fronto-nasal approach. In one patient even a second intervention on the same side was necessary in order to close the leakage in the cribriform plate which was overlooked during the first intervention. Both fistulas had a round, smooth configuration, as is typical of non-traumatic fistulas. There were no signs of bone fracture or fissure in the exposed frontal base of the skull. In spite of the history of head injury in one of our patients, we believe in a non-trau-

Table I *Classification of cerebrospinal rhinorrhea (according to Ommaya et al 1968)*



rhinorrea and pneumatocephalus for more than 3 years

The second case refers to a male of 25 years first seen at the age of nineteen He was born with chondrodystrophy At the age of 15 and 17 he had two minor head injuries without fracture of the skull On admission we observed secretion of a clear watery fluid from his left nostril The chemical analysis confirmed a cerebrospinal rhinorrhea At the ENT Hospital surgery by the fronto-nasal approach on the left frontal base of the skull failed to reveal the cerebrospinal fluid fistula

Scintigraphy and encephalography after insufflation of air showed a hydrocephalus with obstruction of the cerebrospinal fluid circulation in the subarachnoidal spaces and a cerebrospinal fluid fistula into the nasal cavity A localisation of the fistula was not possible. To lower the intracranial pressure a ventriculocardiostomy was performed by the neurosurgeon and the rhinorrhea ceased.

Over the next 2 years the patient developed mental debility and was no longer able to follow the lessons at school He became increasingly somnolent and had to be admitted in the



Fig 1 Chondrodystrophic patient D. S with hydrocephalus spontaneous pneumatocephalus, enlargement of the lateral ventricle brain atrophy and Arnold-Chiari malformation.

-anial bone In the thin areas of the base of the skull this may cause a cerebrospinal fluid leak. Therefore the thin ethmoid is the predilected region of high pressure rhinorrhea. The leak can be considered as a spontaneous healing of hydrocephalus This can be illustrated by a case of Nadjmi (1972) An acoustic neurinoma caused deafness blindness and tetraplegia. After onset of cerebrospinal rhinorrhea 3 years later the patient became able to hear and to walk again The frontal lobes had prolapsed through the destroyed ethmoid into both nasal cavities

This type of spontaneous decompression of the high intracranial pressure by cerebrospinal rhinorrhea was observed in our two cases Chondrodystrophy is known to be a cause of hydrocephalus but the frequency of this combination is unknown. In the skull this leads to a disproportion between the normally growing calotte and the base of the skull with its enchondral ossification This results in a prolongation of the frontal cranial fossa, while the middle and posterior areas of the base of the skull are shortened in all diameters Furthermore we find a premature synostosis of all bony sutures of the base of the skull This may result in a disturbance of the cerebrospinal fluid circulation in the posterior cranial fossa as in our two patients In one case an Arnold-Chiari malformation has been verified Another case of Arnold-Chiari malformation hydrocephalus and cerebrospinal fluid leak is reported by Youngs & Peyton (1953)

High pressure cerebrospinal fluid leaks should be treated in cooperation of neurosurgeons and rhinosurgeons At first the increased intracranial pressure must be lowered by neurosurgical intervention because it is impossible to close the fistula while intracranial pressure is high In our 2 cases this was achieved by ventriculocardiostomy of Pudenz. The second step is to close the fistula, even if cerebrospinal rhinorrhea ceased Our second case demonstrates that drainage of the cerebrospinal fluid may lead to aspiration of air and germs through the fistula. Thus the patients runs the risk of contracting meningitis We think that it is easier and less traumatic for the patient to attempt a closure of the fistula by the rhinosurgical extradural way The fronto-nasal approach has proved to be of use thanks to the adequate exposure of the frontal base of the skull preservation of the olfactory nerves clining of the ethmoid cells and drainage of the sinuses into the nasal cavity The main advantage of the fronto-nasal approach especially in cases of brain atrophy and hydrocephalus is that this vulnerable remaining brain is not touched at all Every neurosurgical intradural approach must lead to injury of this atrophied brain Even in the case of a second rhinosurgical intervention as was necessary in both of our patients the risk for the patient is less than by any intradural approach Both of our patients stand as good examples of cooperation between neurosurgeons and ENT specialists

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We are grateful for his help to Prof Dr Rudolf Kautzky head of the Neurosurgical Department Hamburg University Prof Dr Andreas Thurn head of the Neuroradiological Department, University Hamburg, we would like to thank for the performance of the roentgenography

ZUSAMMENFASSUNG

Bei zwei chondrodystrophen Männern trat im zweiten Lebensjahrzehnt eine nicht-traumatische Rhinoliquorrhoe auf. Als Ursache der Liquorfistel wurde in beiden Fällen ein Hydrozephalus festgestellt. Zunächst wurde der intrakranielle Druck durch eine vom Neurochirurgen vorgenommene Ventrikulokardiostomie gesenkt. Anschließend wurde die Liquorfistel auf rhinochirurgischem extraduralem Wege verschlossen. In Anbetracht der Hirnatrophie beim Hydrozephalus erschien der paranasale Zugang zur Frontobasis als der am besten geeignete und am wenigsten traumatisierende Eingriff

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Table II *Non traumatic cerebrospinal rhinorrhea by increased intracranial pressure*

Author	Year	Cause of hydrocephalus	Localisation of the fistula	Verification
Miller	1876	Unknown since 11th month of life	Cribriform plate	Autopsy
Leber (Locke)	1883	Unknown since birth	Sella	Autopsy
Nothnagel (Locke)	1883	Tumor of lamina quadrigemina	Cribriform plate	Autopsy
Gutsche (Locke)	1895	Pituitary tumor	Ethmoid	Autopsy
Wollenberg (Locke)	1898	Tumor of occipital lobe	Ethmoid and frontal sinus	Autopsy
Meyer (Locke)	1905	Pituitary tumor	Ethmoid rupture of lateral ventricle	Autopsy
Vigouroux (Locke)	1908	Papilloma of IV ventricle	Ethmoid	Autopsy
Souques (Rovit)	1917	Acoustic Neurinoma	Cribriform plate rupture of lateral ventricle	Autopsy
Cushing	19 1	Acoustic Neurinoma	Ethmoid rupture of the lateral ventricle	Autopsy
Barrett	1926	Bilateral acoustic neurinomas	Ethmoid	Autopsy
Locke	1926	Acoustic neurinoma and tumor of the frontal lobe	Cribriform plate	Autopsy
Mankowsky	1929	Pituitary tumor	Unknown	Clinical
Britt	1935	Medulloblastoma	Ethmoid and cribriform plate	Autopsy
Shea	1938	Arachnoiditis	Ethmoid	Autopsy
Som	1940	Pituitary tumor	Ethmoid	Autopsy
MacDonald	1945	Arachnoiditis	Cribriform plate	Clinical
Youngs	1953	Arnold-Chiari malformation	Unknown	Clinical
Menning	1964	Aqueductal stenosis	Sella	Operative
Seeger	1964	Medulloblastoma	Unknown	Clinical
Mukherji	1965	Tuberculoma of the frontal lobe	Cribriform plate	Operative
Rozsíval	1966	Astrocytoma	Ethmoid	Operative
		Meningioma	Ethmoid	Operative
Houdart	1967	Aqueductal stenosis	Ethmoid	Operative
		Aqueductal stenosis	Frontal sinus	Operative
		Arachnoiditis	Ethmoid	Operative
Lehnhardt	1968	Unknown	Ethmoid	Operative
Ommaya	1968	Pituitary tumor ( )	Sella	Operative
		Acoustic neurinoma ( )	Unknown	Clinical
		Pituitary tumor ( )	Unknown	Clinical
		Tumor of III ventricle	Cribriform plate	Operative
		Glioma of cerebellum ( )	Cribriform plate	Operative
Rovit	1969	Cyst of III Ventricle	Ethmoid rupture of lateral ventricle	Operative
		Papilloma of III ventricle	Unknown	Clinical
		Meningioma	Cribriform plate rupture of lateral entricle	Operative
		Aqueductal stenosis	Cribriform plate	Operative
Nadjmi	1977	Meningioma ( )	Ethmoid	Operative
		Acoustic neurinoma	Ethmoid	Clinical
		Papilloma of IV ventricle	Multiple ethmoid	Autopsy
		Acoustic neurinoma	Ethmoid	Autopsy

matic origin of the cerebrospinal rhinorrhea (for both cases)

As shown in Table I non traumatic cerebrospinal rhinorrhea with increased intracranial pressure can be caused by tumor or hydrocephalus From the literature we selected a total of 44 cases of this group (Table II) Tumor is the most frequent cause of non-traumatic cerebrospinal rhinorrhea and the fistulas are predominantly observed in the ethmoid sinus including the cribriform plate It is obvious from this review that the detection of brain tumors is the main diagnostic goal Only 9 of 45 cases of non traumatic cerebrospinal rhinorrhea were due to hydrocephalus and the ethmoid sinus was the site of predilection in these cases too

Long-term increased intracranial pressure leads to enlargement of the cerebrospinal fluid spaces at the expense of the brain and the

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EMBRYONAL RHABDOMYOSARCOMA OF THE MIDDLE EAR

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From the E N T Department Dudley Road Hospital Birmingham, U K

(Received, June 27 1978)

Abstract An embryonic sarcoma of the middle ear occurring in child has been described The mainstay of treatment has been triple therapy The child is well 4 years and 3 months after diagnosis.

Embryonal rhabdomyosarcoma of the middle ear is very rare indeed but in children it is the most common tumour of this region It was first described by Soderberg in 1933 Since then 47 cases have been reported The following is another isolated case

CASE REPORT

R M a 4½ year-old boy presented in the E N T Clinic of the North Staffordshire Royal Infirmary on 16 1 74 with intermittent earache and recent bleeding from the left ear He had had a left aural discharge for years In the past the child had been treated medically for a mild congenital pyloric stenosis

On examination there was a polyp filling the left external auditory meatus The right ear nose and throat were normal The child had a squint for which he was awaiting treatment No abnormality was found in general or systemic examination

On 18 1 74 microscopic examination of the left ear was carried out under general anaesthesia. The entire left external auditory meatus was filled by a pale fleshy friable polyp This was removed revealing a small posterior defect in the pars tensa of the tympanic membrane which did not expose the incus or the head of the stapes.

Investigations

Histological examination of the resected fragments of polyp showed loose embryonic mesenchyme partly covered by attenuated squamous epithelium (Figs 1 and 2) Mitoses were numerous No definite rhabdomyoblasts could be identified on staining with phosphotungstic acid haematoxylin The histological appearance was characteristic of an embryonic sarcoma without maturation toward striped muscle Left Aural Polyp Embryonic Sarcoma.

Tomogram of the mastoid showed sclerosis of the whole of the left mastoid region Radiological examination of the chest did not reveal any metastasis (Haematological examination of the chest did not reveal any metastasis) Haematological examination was normal Left aural swab was sterile on culture

Treatment and follow-up

The tumour field was treated with Cobalt 60 the dosage employed being 5 500 R in 5½ weeks Initially there was no obvious metastatic enlargement of the lymph nodes of neck but after about a week of radiotherapy a node became palpable in the left supraclavicular region Liver spleen and other nodes were not palpable The supraclavicular node was included in the field of radiation

Treatment also included the following cytotoxic agents. Vincristine injection –0 7 mg weekly for 12 weeks. Actinomycin 'D' injection –0 25 mg for 5 days every week for 3 months Endoxana tabs –5 mg orally 3 times daily for 7 days repeated every sixth week.

The child was restless and needed Valium 4 mg orally and 400 mg of Pentothal rectally prior to treatment and injections

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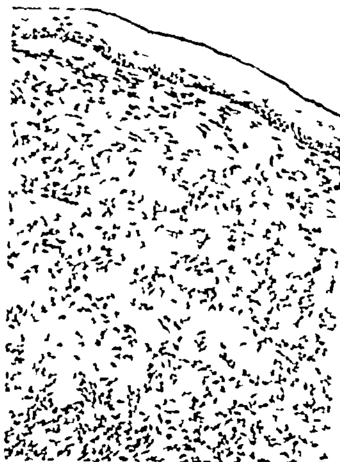


Fig 1 Photomicrograph of L aural polyp showing malignant embryonic mesenchyme covered by squamous epithelium Haematoxylin and eosin ×100



Fig High power photomicrograph showing stellate and strap shaped malignant mesenchymal cells with mitoses beneath squamous epithelium. Haematoxylin and eosin ×400

Syrup Fersamal 10 ml daily was prescribed because his haemoglobin fell to 65% but he was unable to tolerate it He ate well and was gaining weight normally

The tympanic membrane became normal The supraclavicular node became smaller but a tender 1 cm node became palpable behind the left mastoid process

The leucocyte count fell to 900/cm the platelet count was 40000/cm He developed a Proteus Morgani infection of the intestine and a complete alopecia as side effects of cytotoxic therapy

On 3 10 74 the ear was re-examined under general anaesthesia The external canal was normal though expanded The tympanic membrane was intact and myringotomy did not reveal any tumour There was no evidence of recurrence either locally or in the cervical glands

The child was last reviewed on 28 2.78 when the ear was found to be clinically normal and he had normal hearing

DISCUSSION

Rhabdomyosarcoma is a highly malignant tumour arising from the mesenchymal connective tissue About 77 7% of cases occur in children under the age of 12 years and 43.5% under the age of 5 years (Barnes & Maxwell 1972) The tumour occurs most frequently in the urogenital tract and less frequently in the orbit nasopharynx palate and middle ear Approximately 75% of tumours occur in male patients (Horn & Enterline 1958)

Most of the non-botryoid rhabdomyosarcomas arising from the skeletal muscles appear as soft fleshy pink or grey pink but there is

great variation in the size consistency colour and extent of invasion of necrosis The botryoid variety of *rhabdomyosarcomas take* the gross appearance of oedematous often myxomatous polyp having the grape-like form as their name implies This polypoid grape like appearance is due to the non-neoplastic proliferation of the surface epithelium

Not all non-botryoidal sarcomas arise from skeletal muscle e g rhabdomyosarcomas arising from the distal phalanx of thumb and mandible

Horn & Enterline (1958) recognised 4 histological types of rhabdomyosarcoma.

1 Pleomorphic—spindle cell tumour with strap-like cells and brightly eosinophilic cytoplasm *and often with multiple nuclei in tan*dem a number have cross-striations This occurs in adults

2 Alveolar—alveoli separated by trabeculae and lined by cells like epithelium and giant cells with cross-striations This occurs in adults

3 Embryonal—consists of thin spindle cells ending in slim processes a myxomatous matrix may separate the cells some of which contain myofibrils with perhaps cross-striations This occurs in children

4 Sarcoma Botryoids—occurs in relation to mucous membrane they resemble the embryonic variety but *in addition* there is often a concentration of cells immediately beneath the mucous membrane This occurs in children

In general the rhabdomyosarcomas spread *both locally as well as distally* The distant metastasis appears to be a greater threat to cure of the patient with rhabdomyosarcomas The commonest site of distant metastasis is lungs Lymph nodes are also involved but the incidence is not high

When rhabdomyosarcoma occurs in the middle ear it usually has a rapid local spread involving the cranial nerves Systemic and lymphatic spread are often well advanced at the time of clinical presentation On naked eye examination the tumour mimics a chronic suppurative otitis media with a polyp It is therefore very important that all aural polyps especially those occurring in children should be histologically examined

The average survival of embryonic sarcoma of the ear is 7.2 months (Jaffe et al 1971) Much longer survival up to 4? months has been reported by Conte & Sagerman (1971) and Barnes & Maxwell (197?) report a patient alive 1? years after diagnosis As the reported cases were in an advanced state when first diagnosed it is difficult to draw any conclusion as to the effectiveness of treatment. However the general opinion is that surgery radiotherapy and chemotheraphy may materially increase the prospect of survival as shown by *Donaldson et al (1973)*

In our case there was no systemic spread at initial presentation The cervical node enlargement which occurred later was minimal The triple therapy in this case seems to have greatly improved the prognosis The child was last seen in Out Patients about three months ago when he was found well

ZUSAMMENFASSUNG

Ein Fall eines Embryosarkoms des Mittelohres bei einem Kind ist beschrieben Behandlung bestand aus Radiotherapie und drei cytotoxischen Mitteln. *Das Kind* ist wohl 4 Jahre und 3 Monate nach der Diagnose

ACKNOWLEDGEMENTS

I would like to thank M D W Stuart, under whose care the patient was treated to allow me to report this case and for his advice in preparing it, and also Dr B G Ockenden for her valuable suggestions and for preparation of the photomicrograph and reporting the sections.

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ROLE OF THE TENSOR VELI PALATINI MUSCLE IN MOVEMENT OF THE SOFT PALATE

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(Received August 8 1978)

Abstract T xamine the role of the tensor veli palatini muscle in palatal movement, conducted 1) quantitative measurement of palatal movement by selective stimulation of the tensor and levator muscles, and 2) EMG recording of the tw muscles during phonation The results were 1) negligible palatal movement upon tensor stimulation, despite marked velar elevation by levator stimulation and 2) little EMG activity of the tensor and marked EMG activity of the levator during phonation. It was concluded that the tensor plays no role in the palatal function

Of the muscles related to the palate the levator and the tensor veli palatini are regarded as the main muscles involved in the function of the soft palate These two muscles are also thought to have another function, that of dilating the Eustachian tube Our recent work (Honjo et al 1979) however demonstrated that the levator had no direct connection to the tubal function its activity being limited to velopharyngeal closure It therefore seems doubtful after all that the tensor has two activities, in palatal movement and tubal dilation

It is the purpose of this study to re-examine whether the tensor muscle plays any role in palatal function, by utilizing the technique of selective stimulation of the muscle and objective measurement of palatal movement through a force transducer

METHOD

Stimulation of the tensor and the levator muscles was done on 5 dogs Velar movement was evaluated first visually and then objectively by the transducer

After intravenous injection of Nembutal tracheotomy was performed The hamular process of the pterygoid was exposed through a longitudinal incision on the lateral side of the soft palate The tensor which runs cranially from the process was easily identified while the levator was found to lie across the velum about 10 mm dorsal to the process The procedure was performed with little bleeding and minimum damage to the muscles Bipolar hooked electrodes were inserted into both muscles for either electrical stimulation or EMG recording (Fig 1)

As illustrated in Fig. 2 a point on the oral surface of the soft palate was tightly connected to the transducer by a thread The transducer transformed any slight elevation of the palate into an electrical signal as a function of tensing. Palatal movement was measured at two points at its midline which represented the an-

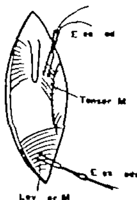


Fig 1 Insertion of the hooked electrodes into the tensor and the levator muscles.

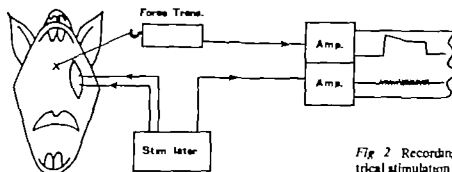


Fig 2 Recording of palatal movement and of the el trical stimulation of the muscles

terior and posterior halves of the palate Then the two muscles were stimulated independently by an electrical current of 100 pulses per second at a voltage of either 5 or 10 volts Thus the degree of velar elevation was recorded simultaneous with the stimulation In addition to palatal movement dilation of the Eustachian tube was recorded in some cases as a sudden drop of ear pressure The procedure was reported in detail previously (Honjo et al 1979)

Second EMG of the two muscles was recorded during phonation (barking)

RESULTS

Visual observation of the soft palate revealed that the levator stimulation produced drastic movement of its posterior half in the dorsocranial direction In contrast tensor stimulation produced no movement of the soft pal other than a slight tonic movement in a limit area near the hamular process

The results of objective measurement by t force transducer were as follows

1 Stimulation of the levator produced marked elevation of the soft palate As show in Fig 3 its posterior half moved drastical in the cranial direction while its anterior h moved slightly (Fig 4) This would seem to l due to the anatomical fact that the levator m through the posterior third of the soft palate

2 Stimulation of the tensor produced no ward movement of the palate at the two poin examined (Figs 5 and 6) From the anatomic point of view the anterior palatal half wou be expected to show some kind of movemen since the tendon of the tensor lies on the pala in the area between the hamular processe

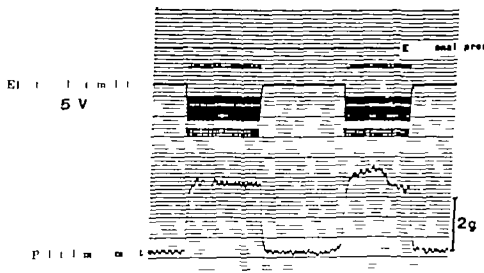


Fig 3 Marked elevation in the posterior half of the palate due to levator stimu lation but no tubal openir

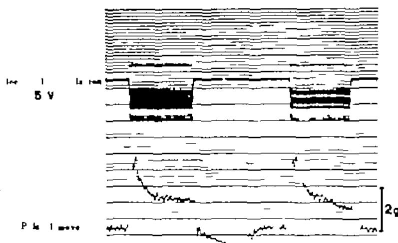


Fig. 4. Slight movement in the anterior half of the palate due to levator stimulation.

However the area failed to show any response such as elevation or depression. On the other hand tensor stimulation produced tubal opening consistently as observed in Figs. 5 and 6.

EMG activity of the tensor and the levator is shown in Fig. 7. During phonation the tensor was found to show slight activity while the levator showed marked activity.

DISCUSSION

Although the tensor and the levator veli palatini muscles are classified as palatal muscles they are regarded also to act in dilating the Eustachian tube. However our recent experimental work (Honjo et al. 1979) failed to demonstrate any effect of the levator upon tubal function, thus indicating that the activity of the levator is limited to velopharyngeal closure alone.

Consequently it seemed improbable that the tensor would have two different functions one for the palate and one for the tube. Our hypothesis was that the tensor had only one major function related to the tube and that its relation to the palate was not significant.

Tröltsch (194?) and Rüdinger (1867) called

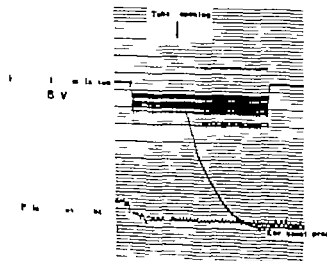


Fig. 5. Tensor stimulation, provoking tubal opening but no movement of the posterior half of the palate.

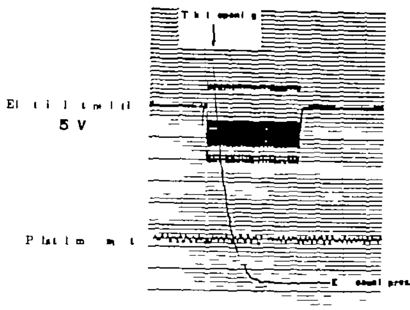


Fig 6 Tensor stimulation provoking tu opening but no movement of its ante half of the palate

the tensor the abductor tubae or dilator tubae Through electrical stimulation of the 5th nerve Rich (1920) found that the tensor exerted no effect upon the soft palate that could be detected by oral examination However the opinion that the tensor contributes to the palatal function by tensing and somewhat depressing its anterior part is still maintaine (Hamilton 1976 Hollinshead 1968) An an tomical study by Körner (1942) and Zöllne (1942) which divided the tensor into two func tional parts—one for palatal function and an other for both the palate and the tube seem to support this opinion

Selective stimulation of the tensor muscle i this study had no effect upon movement of th palate it produced neither elevation nor de pression In contrast to the tensor the levato produced drastic palatal movement as usuall observed during phonation or swallowing Th slight EMG activity of the tensor seen durin phonation agreed with the above finding indi cating the smaller contribution of the tenso to the palatal function

It is concluded that the tensor plays no rol in the palatal function though it is essentia in dilating the tube In consequence we pro pose to call the tensor muscle not the palata muscle but the tubal muscle or more exactly the dilator of the tube as Rüdinger formerly did

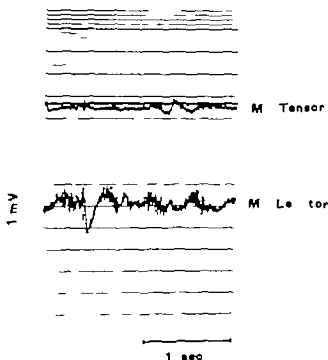


Fig 7 EMG of the tensor and levator during *phonation*

ZUSAMMENFASSUNG

Um die Rolle des M tensor als palatini bei der Gaumen bewegung zu untersuchen wurden 1 die quantitati Messung der Gaumenbewegung durch einzelne Reizun des M tensor oder M levator die EMG-Aufnahm

er beiden Muskeln bei der Phonation ausgeführt. Die Ergebnisse waren: 1. bei der Tensorreizung war die Gaumenbewegung fast nicht nachweisbar trotz der deutlichen Gaumenbewegung durch die Levatorreizung. Deutliche elektromyographische Entladung von M. levator und wenig Entladung von M. tensor bei der Phonation. Es wurde gezeigt daß der M. tensor keine Rolle bei der Gaumenbewegung spielt.

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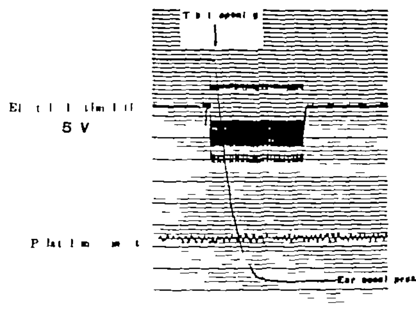


Fig 6 Tensor stimulation provoking t… opening but no movement of its ant… half of the palate

the tensor the abductor tubae or dilator tubae Through electrical stimulation of the 5th nerve Rich (1920) found that the tensor exerted no effect upon the soft palate that could be detected by oral examination However the opinion that the tensor contributes to the palatal function by tensing and somewhat depressing its anterior part is still maintai… (Hamilton 1976 Hollinshead 1968) An a… tomical study by Körner (194?) and Zöll… (1942) which divided the tensor into two fu… tional parts—one for palatal function and … other for both the palate and the tube see… to support this opinion

Selective stimulation of the tensor muscl… this study had no effect upon movement of … palate it produced neither elevation nor … pression In contrast to the tensor the leva… produced drastic palatal movement as usu… observed during phonation or swallowing … slight EMG activity of the tensor seen du… phonation agreed with the above finding i… cating the smaller contribution of the ten… to the palatal function

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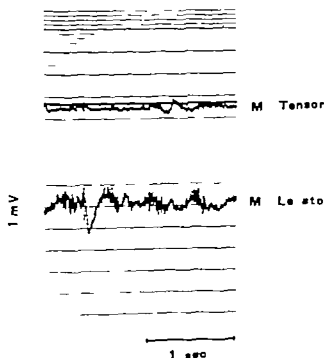


Fig 7 EMG of the tensor and levator during phonation

ZUSAMMENFASSUNG

Um die Rolle des M tensor eli palatini bei der Gau… bewegung zu untersuchen wurden 1 die quantit… Messung der Gaumenbewegung durch einzelne Re… des M tensor oder M levator – die EMG-Aufn…

er beiden Muskeln bei der Phonation, ausgeführt. Die rgebnisse aren. 1 bei der Tensorreizung war die aumenbewegung fast nicht nachweisbar trotz der deut chen Gaumenbewegung durch die Levatorreizung ... eutliche elektromyographische Entladung von M levator nd eng Entladung on M tensor bei der Phonation. urde gezeigt, daß der M tensor keine Rolle bei der aumenbewegung spielt.

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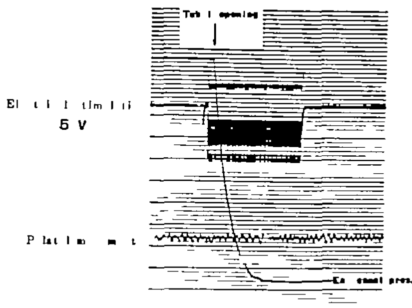


Fig 6 Tensor stimulation, provoking tub opening but no movement of its anterior half of the palate

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Selective stimulation of the tensor muscle in this study had no effect upon movement of the palate it produced neither elevation nor depression In contrast to the tensor the levator produced drastic palatal movement as usually observed during phonation or swallowing The slight EMG activity of the tensor seen during phonation agreed with the above finding, indicating the smaller contribution of the tensor to the palatal function

It is concluded that the tensor plays no role in the palatal function though it is essential in dilating the tube In consequence we propose to call the tensor muscle not the palatal muscle but the tubal muscle or more exactly the dilator of the tube as Rudinger formerly did

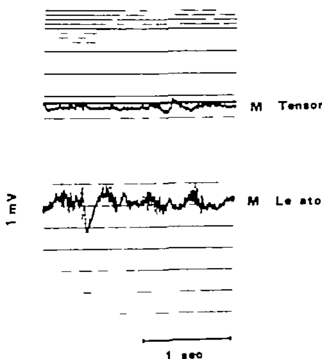


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LARYNGEAL CHONDROSARCOMA IN SWEDEN

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(Received June 1 1978)

Abstract Between 1958 and 1977 5 cases of posterior cricoid chondrosarcoma were reported to the Swedish Cancer Registry These cases and one recently observed case of laryngeal chondrosarcoma are presented in this study of one female and five male patients The tumours grow slowly and recur locally but have not metastasized and none of the pat ents has died from tumour disease The clinical and morphological findings indicate that these tumours represent secondary chondrosarcomas developing from pre-existing benign ecchondromas The reason for the uniformity as to localization is not known The microscopic appearance varied in different parts of the same tumour The presently obtained data and review of the literature indicate that laryngeal chondrosarcomas are extremely rare locally invasive but usually not metastasizing tumours Conservative laryngeal operation is suggested as primary treatment Total laryngectomy is necessary only after local recurrences in olving a major portion of the cricoid cartilage

Laryngeal chondrosarcoma is extremely rare constituting less than 0 1% of all malignant tumours of the larynx (Sirota & Hurwitz 1952 Pohl 1968 Krajina 1975) In 1970 Huizenga & Balogh reported 8 laryngeal chondrosar comas and reviewed 37 cases found in the literature We now present 44 additional cases reported in the literature (Reinhard 1960 Pohl 1968 Kastenbauer & Federspil 1969 Ghalib et al 1969 Al Saleem et al 1970 Hyams & Rabuzzi 1970 Birnmeyer 1971 Lawson et al 1972 Jones 1973 Swerdlow et al 1974 Bryce 1975 Zizmor et al 1975 Krajina 1975 Mitschke 1975 Chambers & Friedel 1976) Thus up to now a total of 81 cases of laryngeal chondrosarcoma have been reported in the literature About 85% of these tumours have affected males usually about 60 years of age and have originated in the pos terior cricoid lamina We also present five additional cases of cricoid chondrosarcoma, not previously reported in the literature They constitute all available laryngeal chondrosarcomas in the files of the Swedish Cancer Registry during the last 20 years To this is added one case recently treated at our hospital

SUMMARY OF CASE REPORTS

During the years 1958 to 1972 five laryngeal chondrosarcomas were reported to the Swedish Cancer Registry and in 1977 still one case was seen at the Department of Otorhinolaryngology University of Umeå, Umeå Sweden The present series consists of these 6 cases 5 men aged 54–65 years and one woman years of age (Fig 1)

The initial main symptoms were dysphonia (4 cases) inspiratory stridor (1 case) and dysphagia (1 case) In all patients indirect laryngoscopy revealed a smooth hard posterior subglottic tumour covered by a normal mucous membrane This was later confirmed by laryngograms and direct laryngoscopy The average duration of symptoms before microscopic diagnosis was about 3 years

All original light microscopic slides were collected and new sections cut from the tissue blocks were stained with haematoxylin–eosin van Gieson s stain and periodic acid Schiff (PAS) At light microscopic study of this material all original diagnoses could be confirmed

A summary of this paper wa read at the XX Nordic Congres on Oto-Rhino-Laryngology Oslo 1978

LARYNGEAL CHONDROSARCOMAS IN SWEDEN 1958 1978

PATIENT BORN	♂ [illegible]	♂ [illegible]	♂ [illegible]	♀ 1905	♂ 1912	♂ 1917
FIRST SIGN OF DISEASE	[illegible]	[illegible]	1957	1955	[illegible]	1972
SYMPTOM	DYSPHONIA	DYSPHONIA	DYSPHONIA	DYSPNEA	DYSPHONIA	DYSPHAGIA
TUMOUR ORIGIN	CRICOID	CRICOID	CRICOID	CRICOID	CRICOID	CRICOID
MICROSCOPIC DIAGNOSIS, YEAR	CHONDROMA, 1963	CHONDRO-SARCOMA 1964	CHONDROMA (with atypia) 1960	CHONDROMA (with atypia) 1957	CHONDROMA, [illegible]	CHONDRO-SARCOMA 1977
RECURRENCE I	1963		1960	[illegible]	[illegible]	
MICROSCOPIC DIAGNOSIS	CHONDRO-SARCOMA		CHONDRO-SARCOMA	CHONDROMA (with atypia)	CHONDRO-SARCOMA	
RECURRENCE II				[illegible]	1971	
LARYNGECTOMY	1963	NO	1961	[illegible]	1971	NO
MICROSCOPIC DIAGNOSIS				CHONDRO-SARCOMA		
RECURRENCE III	1963				1973	
STATUS 1977-78	DIED 1967 INTERCURRENT DISEASE NO TUMOUR RECURRENCE	NO RECURRENCE	NO RECURRENCE	DIED [illegible] INTERCURRENT DISEASE NO TUMOUR RECURRENCE	NO RECURRENCE	NO RECURRENCE

Fig. 1. Summary of data about the 6 cases in the presently reported series. No metastases have been found in these cases and no patient has died from the malignant tumour disease.

Four of the cases were initially chondromas but in two of these there was some suspicion of incipient malignancy. During the following 2 years all these cases showed one or two local recurrences and microscopic signs of true chondrosarcoma (Fig. 2). All tumours originated from the posterior cricoid lamina.

The initial treatment was in all cases local excision, through a laryngofissure or an endolaryngeal approach. Tracheostomy was always done. Radical laryngectomy was performed in four of the cases after one or two local recurrences. Only in one of these cases the microscopic diagnosis of chondrosarcoma seems to have urged a laryngectomy.

At present (June 1978) none of the two non-laryngectomized patients has got a tracheostomy tube. One of them has been free from tumour recurrence for 14 years after the diagnosis of a chondrosarcoma. Small local recurrences were found shortly after laryngectomy in patients. The recurrent tumours were excised *without difficulty*. There have been no lymph node or distant metastases nor any further local recurrences. None of the patients have died from their malignant tumour.

DISCUSSION

Chondrosarcomas are malignant tumours which *originate from* cartilaginous tissue and tend to maintain a cartilaginous appearance during their growth. In contrast to osteosarcomas chondrosarcomas possess no osteoid component (McKenna et al. 1966). This distinction is not only of academic interest but is also of great clinical importance since the treatment and prognosis is quite different for these two kinds of neoplasm (Lichtenstein & Jaffe 1943). A chondrosarcoma usually exhibits a slow progress and metastasizes late in

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the course of the disease whereas an osteosarcoma often presents distant metastases already at the time of the diagnosis (Lichtenstein 1959)

Chondrosarcomas localized to the skeleton are relatively common tumours but only roughly half as common as osteosarcomas Most chondrosarcomas appear in patients 50 to 60 years of age They are only seldom encountered in young people They are slightly more frequent in males than in females Chondrosarcomas seem to originate in all bones which are preformed from cartilaginous tissue Central chondrosarcomas are initially localized to the interior of a bone while peripheral chondrosarcomas develop in relation to the surface of a bone Some of them appear *de novo* and are called primary chondrosarcomas Thus these lesions are malignant from the beginning, and some of these tumours run a fulminant course On the other hand the slow clinical course of many chondrosarcomas, and the roentgenographical macroscopic histopathologic and cytologic characteristics indicate that these chondrosarcomas probably have developed from a pre-existing benign cartilaginous tumour Such lesions are called secondary chondrosarcomas (Jaffe 1961 Barnes & Catto 1966) Secondary laryngeal chondrosarcomas originate from ecchondromas i e chondromas arising in an already existing cartilaginous tissue (cf Barsocchini & McCoy 1968 Hellmich 1969)

Tumours interpreted as secondary laryngeal chondrosarcomas were present in our series (Fig. 1) Like laryngeal chondrosarcomas reported from other countries (Barocchini & McCoy 1968 Huizenga & Balogh 1970) those occurring in Sweden were predominantly cricoid appearing on the endolaryngeal surface of the posterior plate in elderly men The reason for this uniformity as to localization is at present unknown

Another characteristic of laryngeal chondrosarcomas is a slow growth When symptoms appear usually late in the course of the disease these are due to the obstruction of either the airway or the upper digestive tract caused by the bulk of the tumour (Link 1949) A long history of hoarseness dyspnea or dysphagia and a subglottic, non-ulcerating mass originating from the posterior cricoid cartilage suggest a chondrosarcoma The radiographic appearance of chondromas is identical with that of chondrosarcomas differentiation between the two kinds of chondromatous tumours is possible first on microscopic study (Zizmor et al. 1975) The final diagnosis is usually made on material obtained at biopsy (Putney & Moran 1964)

Light microscopically there is no difference between chondrosarcomas of the larynx and those of other parts of the body (O Neal & Ackerman 1952 Goethals et al 1963 Marcove & Huvos 1971) The histopathological criteria of malignancy were defined by Lichtenstein and Jaffe (1943) as follows Many cells with plump nuclei more than an occasional cell with two such nuclei and especially giant cartilage cells with large single or multiple nuclei with clumps of chromatin (Fig 2) These criteria are now universally accepted (Goethals et al 1963 Erlandson & Huvos 1974) However the microscopic diagnosis of chondrosarcoma may be difficult misdiagnoses have been reported to be a problem (Ungerecht 1951 O'Neal & Ackerman 1952 Sirota & Hurwitz, 1952) This seems to be due to the fact that there often is a fairly pronounced variability in the degree of differentiation from one region to the other within one and the same cartilaginous tumour (Al Saleem et al 1970 Dahlin & Beabout, 1971) Therefore when only a biopsy specimen is available to the pathologist chondrosarcomas may be underdiagnosed (Reinhard 1960

Fig 2 Photomicrographs of representative areas of laryngeal chondrosarcomas Note the lobular hypercellularity variation in size and abnormal clustering of anaplastic cartilage cells (Fig ×30), and the plump nuclei (Fig 2b 1000) Binuclear cells are clearly seen in areas of marked degeneration of the intercellular matrix (Fig 2 400) Multinucleated giant cartilage cells are also demonstrated (Fig. 2d 1000) Van Gieson stain

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Chambers & Friedel 1976) The clinical and roentgenographical data should always be considered when making a diagnosis of chondrosarcoma whether localized to the larynx or not

When reviewing cases of laryngeal chondrosarcoma it appears that with the exception of 2 or 3 cases these neoplasms do not metastasize (Goethals et al 1963 Putney & Moran 1964 Huizenga & Balogh 1970 Kurtz, 1975) They are only locally invasive and give rise to local recurrences Thus the biological behaviour of chondrosarcomas in the larynx seems to differ from that of chondrosarcomas in non laryngeal sites Recurrences usually appear first after many years of tumour growth and conservative laryngeal operations can still be employed unless the cricoid cartilage has been extensively damaged

Surgery is the only kind of therapy which should be used for laryngeal chondrosarcomas Irradiation therapy is of no value since this kind of tumour is highly radioresistant Surgical removal of the tumour with preservation of laryngeal function is usually tried in the first instance but becomes more difficult to perform after one or more recurrences (Lawson et al 1972 Jones 1973 Swerdlow et al 1974) Total laryngectomy is necessary only when the entire cricoid cartilage is involved and when any partial procedure would compromise the larynx because of loss of cricoid support

ZUSAMMENFASSUNG

In der Zeit von 1958 bis 1977 wurden fünf Patienten mit hinterem Ringknorpelchondrosarkom zum Schwedischen Cancerregister rapportiert Diese Patienten und ein neulich observierter Fall von Kehlkopfchondrosarkom werden I dieser Studie von einem weiblichen und fünf männlichen Patienten beschrieben Die Geschwülst wachsen langsam und rezidivieren lokal aber sie haben nicht met stasiert und keiner der Patienten ist an seiner Krankheit gestorben Die klinischen und morphologischen Ergebnisse deuten darauf hin daß diese Geschwulste sekundare Chondrosarkome repräsentieren die aus orber ex tierenden gutartigen Ekchondromen entstanden sind Der Grund zu der Gleichformigkeit der Lokalitat ist unbekannt Das mikroskopische Bild variierte innerhalb erschiedener Teile ein und desselben T mors.

Die jetzt erhaltenen Daten und Ergebnisse in der Weltliteratur deuten darauf hin daß Chondrosarkome des Kehlkopfes extrem seltene lokal infiltrierende aber gewöhnlich nicht metastasierende Tumoren sind Konservative Larynxeingriffe sind als primäre Behandlung vorgeschlagen Totale Laryngektomie ist nur nach einem lokalen Rezidiv notwendig das das großere Teil des Ringknorpels umfaßt

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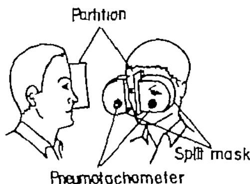


Fig. 1. Split mask with pneumotachometers and adherent airtight, midline partition.

of each half and connected to a Validyne MP45-3 differential pressure transducer. The edges of the mask were fortified with closed cell neoprene foam to facilitate the achievement of a good air seal (Fig. 1). When applied to the face, the halves of the mask were separated by an airtight pliable partition of masking tape which was attached to the mid-line skin from forehead to upper lip with a colostomy adhesive. The efficiency of this barrier to airflow was tested on several occasions by blocking one nasal cavity with petrolatum gauze: when the subject breathed vigorously no airflow was recorded from the pneumotachometer in the obstructed side. In any case, since the pneumotachometer presented a large leak, small leaks between sides of the mask would likely cause little flow between the two sides. The air seal between face and mask was tested immediately before and after each measurement by obstructing the pneumotachometers while the subject attempted to breathe vigorously through the nose. With careful application of the mask, leaks did not present a problem.

Pressure. Trans-nasal driving pressure was measured through a peroral tube to the pharynx by a Statham PM 5 transducer. Subjects were trained to maintain a free airway prior to a series of experiments and this was ensured by observation of a regularly repeating sigmoid trace displayed by an x-y oscilloscope monitor during each period of measurement. It was felt that familiarization of the subject with the technique made an important contribution to the success of the experiment.

Display and recording. Pressure and flow signals were handled by an Electronics for Medicine DR-8 monitor with SGM carrier amplifiers, multitrace and x-y display, and a camera attachment for recording.

Data processing. Amplifier output was transmitted via a buffered interface of our own construction to an analogue to digital converter in an Imsai 8080 A micropressor. Integration of the flow signal every 20 milliseconds and summation gave a printout of minute volume. The product of volume and pressure gave a measure of work. The computer was programmed to allow any number of breaths to be chosen, and to separate inspiratory and expiratory phases. A calculation was included in the computer programme to compensate for the resistance of the pneumotachometers at all anticipated flow rates. Each printout also included time, date, identification of subject and experiment, ambient conditions and breathing characteristics; other relevant data were added as required.

Typical experiment. In a typical experiment the subject was seated at rest, the mid-line facial skin prepared with colostomy adhesive, the partition was shaped for the subject's profile and applied. The two halves of the split mask were then placed and held in position by the subject and the absence of leak confirmed. An oropharyngeal tube was passed between the lips and the x-y monitor observed. When a regularly repeating curve was established, the

Table I. *Nasal breathing parameters at rest with split mask (n=140)*

		$\bar{X} \pm$ S.D.
Minute ventilation	(l min^{-1})	11.3±2.7
Tidal volume	(l BTPS)	0.76±0.26
Respiration rate	(breaths min^{-1})	15.9±4.7

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WORK OF NASAL BREATHING MEASUREMENT OF EACH NOSTRIL INDEPENDENTLY USING A SPLIT MASK

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Abstract The work of nasal breathing was determined in human subjects as a measure of impedance to respiratory airflow The nasal cavities were examined separately and simultaneously with a split mask flow and pressure signals were fed to a microprocessor for on-line computation and printout of respired volumes and work of nasal breathing An alternating resisti e nasal cycle of 3–4 hours duration was demonstrated in the majority of normal resting subjects Reciprocity of the resistive changes in each nasal cavity maintained a constant total nasal respiratory work load of about 0 Joules/litre Moderate changes in breathing rate and tidal volume had little influence on work Inspiratory work was 1 6 times that of expiration Increases in resistance of the dependent nostril were seen when the lateral decubitus position was adopted. Increase in cephalic venous pressure and pathological nasal obstruction increased the work of nasal breathing.

The purposes of this study were (*a*) to examine nasal aerodynamic function in terms of flow resistive work as a measure of impedance to air flow and (*b*) to assess separately and in a non invasive manner the resistive behavior of the nasal cavities and their response to various stimuli In recent years many rhinologists have measured the constantly changing flow resistive characteristics of the nasal cavities in terms of nasal resistance Uncertainty concerning the meaning of resistance ($P/\dot{V}$) as obtained from the marked sigmoid P–V curve (Rohrer 1915) by different observers was avoided in this study by on line computation of flow and pressure signals throughout the respiratory cycle to provide a printout of volume and work (Butler 1960) Some techniques have necessitated the invasion of the nasal cavities with plugs or probes To avoid this a split mask was developed to investigate the two nasal cavities separately and simultaneously without invading the nostrils Many previous workers have shown changes in nasal resistance to occur with apparent spontaneity and also in response to various stimuli such as exercise posture medication and in disease states We have repeated much of this work through a different approach and details of our experiments are presented below

METHODS

Subjects The procedures which were followed were in accordance with the ethical standards of the Committee for Human Experimentation of the University of Toronto. Subjects were selected from student volunteers and laboratory personnel they were classed as normal if they denied nasal symptoms during the preceding three weeks, had no history of chronic nasal disease hay fever serious nasal injury or surgery and showed no evidence of obstructive nasal abnormality on clinical examination Eleven normal subjects were selected Six subjects with nasal obstruction were referred by practicing physicians. The clinical status of all subjects was assessed by an E N T practitioner

Airflow Airflow was measured by means of a split Scuba type mask with a Fleish No 1 or 2 pneumotachometer secured in the face piece

The investigation wa supported by a grant from the Physicians Services Inc Foundation of Ontario Canada

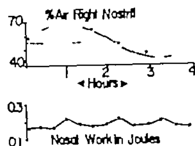


Fig. 2. Nasal cycle as percentage of respiratory air which passes through right nasal cavity. Work of nasal breathing does not fluctuate with cycle but remains at about 0 Joules/litre during 4 hours.

made of nasal work of breathing and distribution of airflow immediately and at five minute intervals.

RESULTS

Results for the pattern of breathing, work of breathing and the nasal cycle were obtained from eleven normal subjects. Measurements were made of ten consecutive breaths every 30 min for four hours, some measurements were repeated and some rejected, which produced a total of 140 useable observations. The effects of changes in ventillation on work of nasal breathing were measured in a second group of twelve normal subjects. In the tables results are expressed as the mean ($\bar{X}$)±one standard deviation and *n* refers to the number of observations, each consisting of a group of 10 consecutive breaths.

Pattern of breathing. The pattern of breathing of normal subjects wearing a split mask and oropharyngeal tube shows some elevation of minute ventilation above expected values of 6–10 litres at complete rest (Cotes, 1965) but is otherwise unremarkable (Table I).

Work of breathing at rest. The work of nasal breathing of the two nasal cavities combined remained constant in individual subjects at rest during periods as long as four hours at 0.20±0.05 Joules/litre (Table II, Fig. 2) and the work of inspiration was 1.6 times that of expiration (Table III).

Work of nasal breathing and ventilation. Work of nasal breathing in the individual subject showed little change with variation in ventilation and breathing rate within the resting range (Fig. 3).

Nasal cycle. An alternating cycle of airflow between the two nasal cavities was clear to visual inspection, as in Fig. 2. In ten of sixteen series of experiments the cycle was of 3–4 hours duration and the most marked fluctuation in airflow showed a ratio of 4 : 1 between the two sides alternately. In three subjects variation in distribution of airflow occurred but an obviously regular cycle was not recognised, in three subjects variation was very slight.

Posture and venous pressure. Change from sitting to supine had no measurable effect on work of nasal breathing or on the distribution of airflow with six normal subjects. On the change to a lateral position from supine, airflow distribution altered to favour the uppermost side—this response was immediate and sustained but varied in magnitude even in individual subjects. On some occasions the lower nasal cavity became almost completely obstructed, as much as 95% of the air passing through the uppermost nostril. The total work of nasal breathing increased in response to lateral posture change but the magnitude of this response was inconsistent also. It was felt that superimposition of the postural response on the nasal cycle could contribute to these irregularities. During the short periods of 20 min in which the postures were maintained the stabilising effect of the reciprocity of the cycle upon total work of nasal breathing was temporarily disrupted.

Table III. *Inspiratory and expiratory work of nasal breathing in normal subjects (n=11)*

	Joules/l ($\bar{X}$ ± S.D.)
Inspired work	0.13±0.04
Expired work	0.08±0.02
Work ratio I/E	1.6

Table II *Work of nasal breathing in normal and obstructed subjects*

		Work in Joules					
		per breath		per minute		per litre	
Subject	*n*	$\bar{x}$	S D	$\bar{x}$	S D	$\bar{x}$	S D
Normal							
PK	13	0.19	0.07	7	0.7	0.19	0.03
SR	11	0.15	0.04	1.5	0.5	0.14	0.03
TP	13	0.20	0.08	4	1.0	0.[illegible]1	0.05
KH	13	0.[illegible]4	0.10	[illegible]3	0.7	0.30	0.08
MI	1	0.08	0.0[illegible]	0	0.6	0.13	0.03
NWe	13	0.10	0.04	[illegible]0	0.7	0.16	0.04
FP	14	0.15	0.06	1.5	0.6	0.18	0.04
RB	13	0.14	0.06	[illegible]8	1.1	0.20	0.05
KM	15	0.15	0.05	[illegible]0	0.6	0.[illegible]4	0.05
NW	13	0.10	0.03	1.8	0.5	0.20	0.03
DC	10	0.[illegible]5	0.04	3.7	0.5	0.28	0.0[illegible]
Total	140						
$\bar{x}$		0.16				0.20	
S D		0.05		0.06		0.05	
Obstructed							
LS	9	0.46	0.25	5.7	[illegible]0	0.49	0.11
JC	11	0.43	0.18	6.4	[illegible]0	0.39	0.08
LL	10	0.[illegible]4	0.08	3.4	1.4	0.40	0.11
GM	13	0.45	0.20	6.8	[illegible]4	0.40	0.08
VN	10	0.37	0.09	4.8	1.3	0.44	0.07
BA	13	0.41	0.11	3.5	0.8	0.63	0.07
Total	66						
$\bar{x}$		0.39		5.1		0.46	
S D		0.07		1.3		0.08	

microprocessor was activated with appropriate commands e g record 10 breaths The V̇ monitor was watched to ensure that all breaths were normal after a suitable interval a printout of (usually) 10 breaths was obtained The mask was again checked for leaks A leak or an abnormal V̇ tracing led to rejection of the result and when feasible a repetition of the observation

Ventilatory pattern and total nasal work of breathing A complete scuba type mask with a Fleish No 2 pneumotachometer in the face piece and an oropharyngeal pressure tube were employed as described above

(*a*) Tidal volume—recordings were made while the subject breathed in time with a metronome at 20 breaths/min^{-} Ten consecutive breaths were recorded at different average tidal volumes from 0 3–2.5 litres The subject attempted to maintain a constant tidal volume with each group of 10 breaths

(*b*) Breathing rate—again the metronome was used for rates which were incrementally increased from 10 to 60 breaths/min After practice the subject was able to approximate an average tidal volume of 750 ml for 10 consecutive breaths Recordings within 5% of the tidal volume were accepted

(*c*) Work load—minute ventilation and work of nasal breathing were recorded while the subject pedalled an ergometer against incremental loading

Posture and venous pressure The subject sat at the head of a stretcher the mask was applied and bilateral flow and work measurements were made on several occasions until the airflow distribution and work of breathing were confidently established Various postures were then assumed and manoeuvres performed which were likely to influence cephalic venous pressure Recording commenced together with the stimulus measurements were

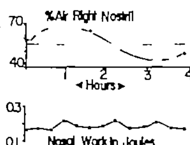


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Table III *Inspiratory and expiratory work of nasal breathing in normal subjects (n=11)*

	Joules/l⁻¹ ($\bar{X}$ ± S.D)
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Expired work	0 08±0.02
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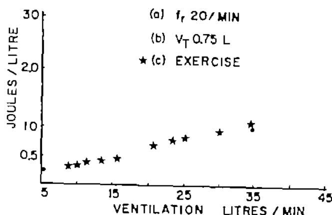


Fig 3 Work of nasal breathing at (*a*) a constant frequency but increasing ventilation (*b*) constant tidal volume but increasing frequency (*c*) increasing ventilation induced by exercise At physiological ventilation rates work is independent of frequency or tidal volume

Unilateral jugular compression or valsalva manoeuver produced no measurable effect whereas bilateral jugular compression and head hanging over the edge of the stretcher produced a marked increase in work of breathing in some instances it was more than doubled but there was no change in distribution of airflow Recovery to the previous resting state accompanied cessation of the manoeuvre and was almost immediate Response and recovery were sufficiently rapid to be demonstrated in a 10 breath observation period which occupied less than one minute

Vasoactive medication Xylometazoline 0 1% abolished the nasal cycle reduced total nasal work of breathing from a mean of 0 18±0 02 to 0 05±0 02 Joules/litre (p<0 05) and the distribution of airflow coincided with the arithmetic mean of the observations taken over a proceeding lengthy period in each of six subjects tested Metacholine abolished the nasal cycle and increased the work of nasal breathing in each of 2 subjects

Nasal obstruction Six patients with clinical nasal obstruction showed increased work of breathing at rest (Table 2) and in four of the patients obstruction was due to mucosal swelling their work of nasal breathing was reduced to the normal range after local application of Xylometazoline 0 1%

DISCUSSION

The curvilinear relationship between pressu and flow of respiratory air as it passes throu the nose was recognised by Rohrer in 191 and since that time many observers have mad use of this aerodynamic relationship to quant fy nasal obstruction to airflow In general th most common nasal airway resistance meth ods either approximate the *P V* relationsh throughout the respiratory cycle or select th relationship at a particular point of pressure o flow rate In neither case has a consisten method been followed by different observer (Kern 1978 Cole 1976)

We have chosen to portray nasal impedanc to respiratory airflow in terms of work o breathing (Butler 1960) We feel that this pre cise summation of *P V* relationships through out the laminar and turbulent portions of the cycle during tidal breathing is unambiguous and allows estimation of flow resistive work throughout tidal breathing thus providing a useful assessment of impedance to airflow through the nose Work is a clearly defined term in this context as distinct from resist ance it has meaning in the physiological eco nomy of the body and it may be used to com pare aerodynamically different airways It should also be noted that use of the work of breathing avoids a problem raised by resist ance measurements at a given flow rate when utilizing the split mask For instance if a cer tain flow rate is used for the whole nose what appropriate rates should be chosen for the separate nostrils? Konno (1969) who em ployed a split mask calculated resistance at a low flow rate of 0 1 litres/sec i e he used the more linear portion of the curve where the *P V* relationships are simple However most of the respiratory airflow takes place in the re gion of turbulence (Knapp et al 1970)

The two nasal cavities were examined sepa rately and simultaneously without invading either with a plug or a probe (Konno 1969) The disadvantages of invading the nasal ca vities are shown by the increases in resistance which follow application of sli ht stimuli such

as saline air jets cotton pledgets or even a nasal speculum (McLean 1976) Each side normally manifests a constantly changing flow resistance brought about by vasoactivity and the degree of distension of the capacitance vessels of the nasal mucosa especially of the inferior turbinates (Stoksted, 1953 Malm 1974 Eccles, 1977)

Clinical observation and various methods of resistance measurement have demonstrated the cycling congestion and decongestion in each nasal cavity and its alternation with the opposite side since it was noted by Kayser almost a century ago The two cycles may become irregular out of phase or otherwise disrupted by disease postural change altered venous pressure vasoactive medication, allergens or other irritants (Ogura & Stoksted 1958) The manifestations were clearly verified by our technique which demonstrated ample sensitivity for detection and measurement of the flow resistive changes We were also able to show a marked difference in energy expenditure between respiratory phases at rest, inspiratory flow resistive work was greater than that of expiration a feature which is not evident from a study of the literature

We demonstrated the separate flow resistive changes of the nasal cavities in terms of distribution of airflow and found them to be reciprocal in the majority of normal subjects combined flow resistive work remained constant at 0 ?±0 05 Joules/litre over periods of several hours in normal individual subjects at rest The stable level of the combined work of nasal breathing is maintained although the distribution in airflow between the two nasal cavities is constantly changing, because these changes are reciprocal An increase in obstruction to airflow through one side is accompanied by a corresponding decrease in the other Furthermore total nasal work of breathing shows little change with the moderate changes in ventilation of the resting subject (Fig. 3) Although both hyperventilation and exercise bring about changes in nasal resistance (Richerson and Seebohm 1968 Dallimore & Eccles 1977) these were not evident in the ventilation ranges we employed All three curves have a similar small inclination at resting levels With increasing exercise the work of nasal breathing increases until mouth breathing occurs.

In healthy subjects at rest the magnitude of the work of nasal breathing determined by our methods is 0.2 Joules/litre by Butler s (1960) methods 0.3 Joules/litre and by his calculations which employed the formulae of Otis Fenn & Rahn (1950) 0.26 Joules/litre The work of nasal breathing of about 0.2–0.3 Joules/litre found by Butler (1960) and ourselves exceeds the flow resistive work of the other airways combined during mouth breathing This latter figure as obtained by several observers (Butler 1960 Hedstrand 1969 Ballantine et al 1970) is about 0 1–0.2 Joules/litre A change from mouth to nasal breathing at rest therefore increases total flow resistive work from 0 1–0 2 Joules/litre to 0.3–0 4 Joules/litre a factor of two The addition to the nasal passages of the remaining flow resistive components of the extra thoracic airways (trachea, larynx pharynx) establishes respiratory airflow resistance as mainly extra thoracic and its proportion increases with increasing ventilation The large resistive difference between the pulmonary and extra thoracic airways is due in part to turbulent air flow in the latter which causes an exponential increase with increasing flow rate whereas the streamlined pulmonary airways with their enormous combined cross-sectional area and their slow and more laminar air flow provide much less resistance Furthermore dynamic luminal changes of the major resistive segments of the extra thoracic airway (larynx nose and mouth) augment the amplitude of their resistive contribution

The present writers believe that the series of experiments outlined above indicate that the techniques they have described and employed provide a sensitive measure of nasal impedance to respiratory air flow The results we have obtained are consistent with clinical and instrumental observations of the past and the

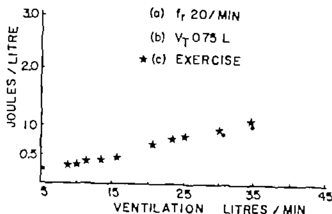


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ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die Arbeit von Nasenatmung wurde bei Menschen als eine Messung von Widerstand gegen den Atmungsluftfluß festgesetzt Die Nasenhöhlen wurden separat und gleichzeitig mit einer geschnittenen Maske betrachtet Fuß und Drucksignale wurden einem Mikroprozesser übermittelt für augenblickliche Ausrechnung und Abdruck der At mungsvolumen und der Nasenatmungsarbeit Ein alter nierender widerstandsfähiger Nasenzyklus von drei bis vier Stunden zeigte sich bei der Mehrheit von normalen ruhenden Menschen Wechselwirkung der widerstandsfähigen Wechsel jeder Nasenhöhle hielt eine unveränderte ganzliche Nasenatmung von um 0,_ Joules/l Mäßige Wechsel der Atmungshäufigkeit und des Atemzugvolumens hatten kleinen Einfluß auf die Arbeit. Einat mungsarbeit war 1 6 mehr als die Ausatmungsarbeit Die Vergrößerungen in der Widerstandsfähigkeit des abhängigen Nasenlochs sah man wenn das Individuum auf der Seite lag Vergrößerung im venosen Druck im Kopf und pathologische Nasenhinderung vergrößerten d e Arbeit der Nasenatmung

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The authors would like to express their gratitude to Oscar Fastag for constructing the computer interface and writing the computer programs.

ZUSAMMENFASSUNG

Die Arbeit von Nasenatmung wurde bei Menschen als eine Messung von Widerstand gegen den Atmungsluftfluß festgesetzt Die Nasenhöhlen wurden separat und gleichzeitig mit einer geschnittenen Maske betrachtet Fuß und Drucksignale wurden einem Mikroprozesser übermitteh für augenblickliche Ausrechnung und Abdruck der Atmungsvolumen und der Nasenatmungsarbeit. Ein alternierender widerstandsfähiger Nasenzyklus von drei bis vier Stunden zeigte sich bei der Mehrheit von normalen ruhenden Menschen. Wechselwirkung der widerstandsfähigen Wechsel jeder Nasenhohle hielt eine unveränderte ganzliche Nasenatmung von um 0.2 Joules/l Maßige Wechsel der Atmungshäufigkeit und des Atemzugvolumens hatten kleinen Einfluß auf die Arbeit. Einatmungsarbeit war 1 6 mehr als die Ausatmungsarbeit Die Vergrößerungen in der Widerstandsfähigkeit des abhängigen Nasenlochs sah man wenn das Individuum auf der Seite lag. Vergrößerung im venosen Druck im Kopf und pathologische Nasenhinderung vergroßerten die Arbeit der Nasenatmung

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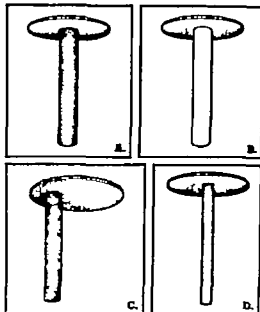
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TORP[1] Implants
(Total Ossicular Replacement Prothesis)

A. Shea[1] TORP Implant
Used to replace the entire ossicular chain. Placed from the oval window to the drum. Broad surface prevents tilting and distributes pressure over a larger area.

B. Shea[1] Plasti Pore and Teflon[2] TORP
Designed for cases in which a tissue-inviting material is needed beneath the drum and a solid Teflon[2] shaft in the oval window. The flange is Plasti-Pore.
[1]Made for John J. Shea, M.D. Memphis, Tennessee

C. Austin[2] Off-Centered TORP
Modification of the Plasti-Pore columella provides a direct—rather than angled—union between footplate and prosthesis.
[2]Made for David F. Austin, M.D. Chicago, Illinois

D. Causse[3] Modification of Shea TORP
Offers the same unique features of the basic columella design, but with a slimmer shaft.
[3]Made for Jean B. Causse, M.D. Beziers, France

PORP Implants
(Partial Ossicular Replacement Prothesis)

A. Richards PORP[4]
Designed to replace the ossicular chain from the drum to the stapes. Hollow post accommodates the stapes, and broad-faced flange interfaces the tympanum.
[4]Made for Ralph Caparosa, M.D. Pittsburgh, Pennsylvania

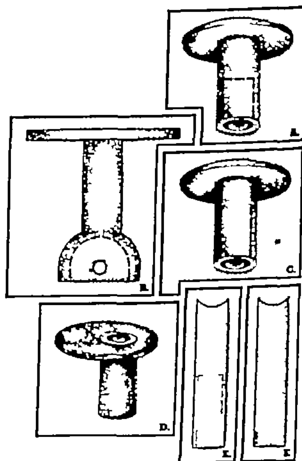
B. Robinson[®] PORP[5]
Features an elliptical head which helps to deter impingement on the posterior auditory canal. Socket-type cup seats on the stapes superstructure.
[5]Made for Mendell Robinson, M.D. Providence, Rhode Island
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C. Modified Plasti Pore PORP[6]
Offers visibility of the stapes superstructure through the cannulated stem.
[6]Made for Leslie J. Bieck, M.D. Chicago, Illinois

D. Off-Centered Plasti-Pore PORP
Off-center position of shaft provides direct (rather than angled) stapes-to-tympanum placement. Cannulated shaft allows visibility of stapes during surgery.

E. Grate[7] Malleus-To-Stapes Strut
Radiused upper portion conforms to the shape of the malleus. Hollow post seats on the stapes superstructure.
[7]Made for M. R. Grate, M.D. Tallahassee, Florida

F. Shea[8] Plasti Pore Strut
Radiused portion fits the shape of the malleus. Portion that rests on the footplate can be trimmed for a more conforming fit.
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Plasti-Pore Piston with Armstrong-style Platinum Ribbon Loop or Stainless Steel Wire Loop

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B. Plasti-Pore Bobbin Drain Tube

The combination of the bobbin design and Plasti Pore material results in myringotomy tube for long-term ventilation. Teflon lumen helps to prevent tissue in growth and clogging of the lumen itself.

C. Armstrong* Teflon* Plasti-Pore Drain Tube

The Armstrong Teflon Drain Tube is modified in this version with Plasti Pore sleeve encasing the lumen. Beveled flange is Teflon.

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D. Plasti-Pore Reconstruction Disc

Plasti Pore discs of varying sizes provide tissue-sorting replacement for tissue voids. Especially suited for radical mastoid surgery to restore contour.

a. b. c. d.

TORP Implant

PORP Implant

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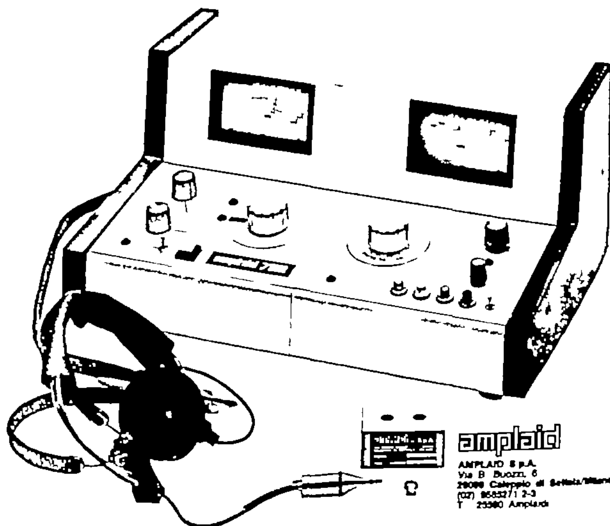
entirely linea calib tion of put/output function over the entire complianc range (0 to 5 cc)

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a Oto-Laryngologica Vol. 88 1979 (Article by Ornitz *et al.*).

ge 251 Table 4

e column headings were misplaced and there were also two errors on line 3 m the bottom The corrected table should read

ble IV *Nystagmus parameters representing the time course of the response from the begin g of acceleration to the end of secondary nystagmus*

	Correlation with $\log_{10}$ age (or age)[1] r[a]	Mean ± 1 S.D.	
		23 infants[d]	23 children[e]
ak (maximum) value of slow component unit splacement, *primary* nystagmus			
Displacement (deg per beat)	−0.35[a] (−0.43)[b]	50.6±17.1	39.0±15.6[c]
Latency from beginning acceleration (sec)	0.47[b]	12.0±4.0	16.7± 6.3[b]
ak (maximum) value of slow component elocity *primary* nystagmus			
Velocity (deg/sec)	−0.20	90.9±32.7	76.6±16.0
Latency from beginning acceleration (sec)	0.43[b]	14.0±2.0	15.7±1.7[b]
tency beginning acceleration to end f *primary* nystagmus (sec)	0.58[c]	34.4±5.8	40.0±6.6[b]
lapsed time from end of *primary* to beginning of *secondary* nystagmus (sec)	0.53[c] (0.46)[b]	3.9±3.5	8.5±4.5[c]
tency beginning acceleration to start of secondary nystagmus (sec)	0.75[c]	38.2±5.9	48.5±7.4[c]
ak (maximum) value of a slow component unit displacement, *secondary* nystagmus			
Displacement (deg per beat)	−0.32[a] (−0.41)[b]	33.2±14.8	27.2±10.7
Latency from beginning acceleration (sec)	0.56[c]	67.9±12.1	92.1±18.7[c]
eak (maximum) value of slow component velocity *secondary* nystagmus			
Velocity (deg/sec)	−0.48[b]	38.3±21.1	19.2±6.0[c]
Latency from beginning acceleration (sec)	0.49[c]	68.1±16.9	98.1±32.4[c]
atency from beginning acceleration to end of *secondary* nystagmus (sec)	0.71	125.1±23.3	174.1±23.2[c]

[1] The linear correlation coefficient was calculated after log transformation of age except in those instances wh ection of the scattergrams suggested linear relationship between the nystagmus parameter and age The majority nystagmus parameters in this table showed an exponential relationship to age.

The probability that is significantly different from zero (two-sided test) is: [a] <0.05 [b] <0.01 [c] <0.001

Under 14 months old

Over 2 years old.

The probability that the differences between the means of the infants and those of the children are significant, usi two-sample *t*-test with separate variances and an approximate degree of freedom solution (two-sided test) is: <0.05 <0.01 <0.001

age 253 line 12

The word changes" should read "change

age 255 line 12

The word "no" should read "to"

age 256 6th reference

Hornrubia should read "Honrubia"

Acta Otolaryngol 88. 155–160 1979

AUDIOLOGIC FINDINGS AFTER STEREOTACTIC RADIOSURGERY IN NINE CASES OF ACOUSTIC NEURINOMAS

Anita Hirsch Georg Norén[2] and Henry Anderson

From the [1]Department of Audiology and the [2]Department of Neurosurgery Karolinska Sjukhuset Stockholm, Sweden

(Received October 17 1978)

Abstract. Nine cases of acoustic neurinoma were treated by stereotactic radiosurgery between 1969 and 1974 The follow-up period can now be regarded as sufficiently long for preliminary evaluation of the results An arrest of growth or shrinkage of the tumour was observed in [illegible] of the 9 cases. In one case open surgery was performed 2 years after irradiation and histological examination showed regressive changes of the type expected after radiation Audiological examination revealed that in the majority of cases radiosurgery could be performed without causing serious damage to the hearing function The average hearing loss present before treatment increased on average only 20 0 dB in the most successful 7 cases None of the patients suffered facial nerve involvement In small and medium-sized acoustic tumours this method offers a satisfactory therapeutic alternative worthy of consideration.

Anatomical experience and improved techniques in oto- and neurosurgery have made it possible to carry out total removal of acoustic neurinomas with low mortality rate and in the majority of cases with a functional preservation of the facial nerve and in a few cases also of hearing ability At the same time advances in audiology and radiology have made it possible to make an early diagnosis before the acoustic nerve has suffered severe damage and at a stage when the tumour still is likely to be small and removal comparatively easy

In his series of 200 cases House (1968) reports the results in 24 cases of small and medium-sized tumours operated on with the middle fossa approach The hearing was reported saved' in 7 cases and in 14 the facial nerve function was also preserved Glasscock et al (1978) in tumours of the same size and with the same surgical approach succeeded in maintaining some hearing in 6 out of 10 specific attempts. The authors report good facial function in one of these cases and no facial weakness in another which has to be interpreted as some degree of facial nerve dysfunction in the remaining 8 cases. Yasargil et al (1977) and DiTullio et al (1978) report preserved facial nerve function in about 80% of cases of small and medium-sized acoustic tumours (30 and 34 cases respectively) removed by microneurosurgery· in 2 cases the hearing was preserved With respect to preserved facial nerve function good results have been reported with the translabyrinthine approach (Palva et al 1978) but as the organ of hearing is here intentionally sacrificed this method is of little interest in connection with the present study

Even under the best conditions open intracranial surgery of all descriptions is accompanied by a certain risk of serious complications As long as surgical removal of acoustic tumours irrespective of approach seems in the majority of cases likely to cause total or severe impairment to hearing and involves a not insignificant risk of facial nerve dysfunction, the search for alternative treatment procedures is desirable

This paper deals with the results in the first nine cases of acoustic neurinomas treated by stereotactic radiosurgery (Leksell 1971*a*)

METHODS

Audiological investigation included pure tone audiometry speech discrimination test, Fow

This investigation was supported by research grant from Stiftelsen Tysta Skolan Stockholm, Sweden.

Table I *The result of the preoperative audiological tests and ENG examinations of the nine rr ated cases*

Case no.	Age	Thresh (dB)*	Discr score (%)	Fowler's test	Tone decay	Stapedius reflex test Thresh.	Decay	Caloric response
1	34	55	70	Recr	Normal	Elevated	(*)	Reduced
2	47	53	70	Recr	Normal	Elevated	Normal	Reduced
3	55	32	64	No recr	Abnormal	Elevated	(*)	Absent
4	44	28	100	Recr	Normal	Elevated	Pathol.	Normal
	49	20	96	Recr	Normal	Elevated	Pathol	Reduced
6	57	45	78	Recr	Normal	Elevated	Pathol.	Reduced
7	55	35	58	No recr	Abnormal	Elevated	(*)	Absent
8	40	42	2	Recr	Abnormal	Elevated	(*)	Reduced
9	61	53	40	No recr	Normal	Elevated	Pathol.	Normal

*Thresh Tone threshold given as mean of test frequencies 0.5 1 and 2 kHz.
Test not applicable due to elevation of reflex threshold.

The aim when planning the treatment was to irradiate the periphery of the neurinoma with approximately 50% of the central dose 50 to 100 Gy in this series This was accomplished by making the periphery coincide as closely as possible with the steepest part of decline of the radiation field thus minimizing the dose to structures in the vicinity In order to fulfil these demands tumours of a size not exceeding about 1.5 cm were preferred for this series This limitation refers to the gamma unit I which was taken into use in 1967 Gamma unit II (introduced 1975) which has a larger almost spherical field of radiation, was used for re-irradiation in two of the cases.

By using plain X-rays pneumoencephalograms and in some of the cases metrizamide cisternograms (Grepe 1975) the exact localization and extension of the tumour was determined

MATERIAL

Nine cases of acoustic neurinomas, 5 women and 4 men (age range 21 to 61 years) were treated during 1969–74 in gamma unit I Three tumours were left-sided and 6 right-sided In one patient (case no 1) with Recklinghausen s disease and bilateral tumours, the smaller tumour on the right side was treated in gamma unit I and the contralateral tumour later treated in gamma unit II. In 7 cases the primary symptom was hearing loss and/or tinnitus one patient complained of vertigo and one showed trigeminal symptoms

The results of preoperative audiological tests and ENG examinations are shown in table I From the table it is evident that conventional tests of speech discrimination, ABLB and tone decay test had low diagnostic value in contrast to stapedius reflex measurements which identified every case.

RESULTS

The follow-up period after irradiation is now 4 to 9 years. In Table II hearing function pre-

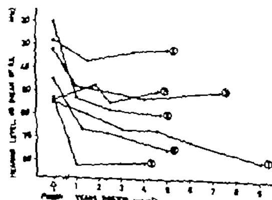


Fig 3 Preoperative tone threshold values and postoperative course of hearing in 7 of the 9 irradiated cases (case nos. in circles) Excluded are case no. 2, which was submitted to open neurosurgery followed by total deafness, and case no 8 where the irradiation hit the tumour excentrically (see text).

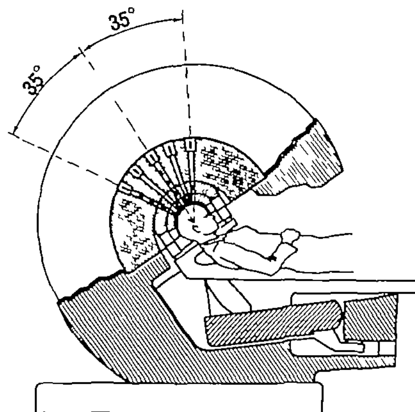


Fig 1 The gamma unit

ler test (ABLB) tone decay test and stapedius reflex measurements including reflex decay test The reflex measurements were made using the technique described by Anderson (1969)

The Fowler test was regarded as positive if an imbalance of 15 dB or more was recorded (mean at 0.5 1 0 and 2 0 kHz or applicable test frequencies from these) In the threshold decay test the corresponding requirement was a threshold shift of more than 25 dB at 2 0 kHz or more than 15 dB at 1 0 kHz The stapedius reflex threshold was regarded as elevated if more than three of the six test frequencies outranged that of normal distribution (Anderson & Wedenberg 1968) Reflex decay was classified pathological when the *half life* of the response amplitude was below 5 sec on stimulation with 0 5 and 1 0 kHz 10 dB above the individual reflex threshold (Anderson *et al* 1970)

The tumours were irradiated according to the technique described by Leksell (1951 1971*b*) by 179 gamma radiating ^{60}Co sources arranged as a part of a sphere (Fig 1) All beams run through a system of collimators cross-firing at the centre of the sphere giving rise at this point to a very well-defined field of radiation characterized by an extremely steep dose gradient (Fig 2) By means of a fixation device attached to the head the patient was positioned in the apparatus in such a way that the field of radiation coincided exactly with the target point the tumour

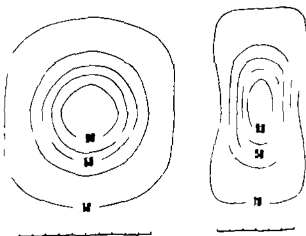


Fig 2 Frontal (*left*) and sagittal sections of the dose distribution of the gamma unit 1 The numbers indicate the relative dose in per cent Scale in mm

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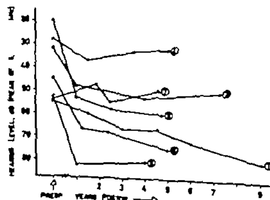


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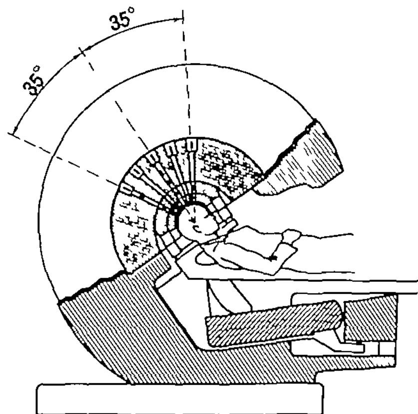


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Fig 2 Frontal (*left*) and sagittal sections of the dose distribution of the gamma unit I The numbers indicate the relative dose in per cent Scale in mm.

:	Facial nerve loss	Side effects	Return to normal life
	N	No	Yes, hearing aid on op. ear
	No	No	Yes
s	No	N	Yes
	No	Tingling in the cheek	Yes
yr	No	N	Yes, hearing aid on op ear
yr	No	N	Yes
rr	No	No	Yes
rr	No	No	Yes

1 3 5 7 and 9) In 2 cases (nos 4 and 8) there was an initial growth which was arrested by further irradiation During the first year of observation one neurinoma showed an enlargement from 9 to 10 mm after which the growth ceased (case no 6)

The 8 cases treated by stereotactic surgery only are unlikely to require further treatment, the patients have resumed a normal life and none have a facial nerve involvement

DISCUSSION

The open surgical treatment of acoustic neurinomas has in the hands of a limited number of skilled oto- and neurosurgeons reached a high degree of perfection Radical removal is the rule even in the case of large tumours The facial nerve function can often be preserved However with respect to maintained hearing function the outcome seems rather hazardous despite anatomical preservation of the cochlear nerve

The results in the present series show that it is possible to treat acoustic neurinomas by stereotactic radiosurgery with arrest of growth and even shrinkage of the tumour The experience gained in this first study indicates that this method offers a promising alternative to traditional surgery in cases of small and medium-sized acoustic tumours In contrast to the results of open surgery this can be achieved without risk of damage to the facial nerve and with a high probability of preserved hearing. True enough some deterioration in hearing threshold is generally seen during the first years after irradiation but after that the hearing level in most cases shows a clear and promising tendency towards stabilization or even a slight improvement. In one case (no 7) this improvement in threshold seems to have occurred without preceding impairment.

The postoperative speech examination in this series indicates an average reduction of discrimination score of 34% It is, however not possible to say whether this figure reflects the true tendency as the individual postoperative scores were too scattered to fit into a general pattern.

It is interesting to note some changes in the stapedius reflex recordings that appeared in three of the patients after irradiation (cases 3 4 and 7) The follow-up examinations showed here a tendency towards successive normalization of both reflex threshold and reflex decay a process which in the extreme case 3 led to a complete recovery of reflex threshold and reflex responses with complete absence of reflex decay As in this case the recovery in reflex status was paralleled with a normalization also in the ABLB test, the audiometric pattern here postoperatively changed into that of a cochlear impairment

CONCLUSIONS

The follow-up period can now be regarded as sufficient for a preliminary evaluation of ste

Table II *Tumour size and hearing function pre and postoperatively*

	Preop.		1–2 yr postop			4–5 yr postop		Present status	
Case no	Thresh. Discr	Tumour size (mm)	Thresh Discr	Tumour size (mm)	Further treatment	Thresh Discr	Tumour size (mm)	Thresh. Discr	Tumour size (mm)
1	55 dB 70%	12	60 dB –	12	–	67 dB 0%	10	80 dB –	10
2	53 dB 70%	30	63 dB 62%	30	Open surgery	–	–	–	
3	32 dB 64%	24	48 dB 60%	23	–	53 dB 48%	18	50 dB 46%	11
4	28 dB 100%	~30	37 dB 88%	~30	Re irrad	35 dB 90%	27	3 dB 90%	27
5	20 dB 96%	14	53 dB 80%	11	–	60 dB 26%	9	60 dB 26%	9
6	45 dB 78%	9	68 dB 40%	10	–	75 dB 38%	10	75 dB 38%	10
7	55 dB 58%	13	48 dB 68%	11	–	50 dB 56%	8	50 dB 56%	8
8	42 dB 2%	10	Deaf	14	Re-irrad	Deaf	13	Deaf	13
9	53 dB 40%	9	82 dB 26%	–	–	81 dB 10%	7	81 dB 10%	7

"Thresh" Tone threshold given as mean of test frequencies 0.5 1 and 2 kHz. Discr Denotes discriminant score in speech test.
* Intracapsular removal performed one year before gamma irradiation.

and postoperative is expressed as tone threshold (mean 0 5 1 0 and 2 0 kHz) and speech discrimination score the occurrence of neurological complications is also stated Seven of the 9 cases have retained hearing function including ability to discriminate speech One of them (no 1) claims to benefit from a hearing aid in the treated ear although no discrimination can be demonstrated in the speech test These 7 cases showed between preoperative examination and follow up on average an increase in tone threshold of 20 0 dB and a mean reduction in the discrimination score of 34% The most pronounced change in tone thresholds was recorded between preoperative examination and the first follow-up at 1 to 2 years After that period the threshold sensitivity stabilized or showed a much slower decline in 3 cases the initial drop was followed by a slight improvement in threshold (Fig 3) With respect to discrimination scores the picture is too inconsistent to serve as basis for an evaluation of the tendency

Two cases suffered total loss of hearing case 2 in connexion with surgical removal of the tumour (see below) and case 8 where positive cisternography at one year follow-up revealed that the radiation field had been excentrically placed in the tumour In case number 2 the patient primarily refused open surgery Radiosurgery was therefore attempted Two years later when there had been no decrease in tumour size the patient consented to operation Histological examination of the removed tumour tissue showed regressive changes of the type expected after irradiation Surgery resulted in deafness and a total facial nerve loss

Table II also gives information on the size of the tumours and it is evident that 5 out of 9 tumours diminished after radiosurgery (cases

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HAIR CELL CONDITION AND AUDITORY NERVE RESPONSE IN NORMAL AND NOISE-DAMAGED COCHLEAS

M C Liberman and D G Beil

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(Received October 10, 1978)

Abstract Histological and physiological data are presented from cats born and raised in a low-noise environment and from cats with long-standing, noise-induced threshold shifts Even after 1½-year survival from acoustic trauma, there were threshold elevations of roughly 40 dB in the response of single auditory-nerve fibers which could not be correlated with significant loss of hair cells An attempt was made to correlate these threshold differences with differences in the condition of the sensory cells as seen in light-microscopic examination of epon-embedded surface preparations. Of all the histological features evaluated the orderliness of the stereocilia, on both inner and outer hair cells, showed the closest correlation with single-unit thresholds In the final analysis most of the threshold shift in the noise-exposed ears could be accounted for by loss or damage to sensory cells clearly visible under the light microscope

In the search for the structural bases of noise induced permanent threshold shift most work has been focused on the sensory cell population in the cochlea It has been noted that under some circumstances there can be significant noise induced threshold shift without any significant loss of sensory cells (Hunter Duvar & Elliott, 1972 and 1973 Ades et al 1974 Liberman & Kiang 1978 Ruggero 1978) One interpretation of this finding is that there has been sublethal damage to the sensory cells remaining in the cochlea. Anatomical studies at the ultrastructural level have made it clear that there are noise-related alterations in the condition of those hair cells which remain after acoustic trauma (Engström Ades & Bredberg 1970 Spoendlin 1971 Lindemann & Bredberg, 1972, Wersäll 1973)

Some of the structural changes described in the electron microscope can be seen in a light microscopic examination of a "surface preperation" of the cochlea (Engström Ades & Andersson 1966) As was pointed out by Engstrom et al (1966) careful examination of hair cells in the light microscope can provide much information about the condition of the stereocilia the nucleus and the cytoplasm and about the distribution of lysosomes and even mitochondria. Although a light microscopic analysis has the obvious disadvantage of significantly lower resolution only with the light microscope is it feasible to study the entire cochlea and thus make a systematic study of any hair cell pathologies.

The aim of the present study was to make such a systematic study of the condition of hair cells remaining in ears with permanent threshold shift associated with minimal hair cell loss Threshold shift was evaluated at the level of single-unit responses in the auditory nerve.

METHODS

The animals used in this study included 6 cats born and raised in a low-noise chamber (Liberman 1978) and 9 routine cats (obtained from local animal suppliers) exposed to acoustic trauma. All of the routine animals weighed less than kg at the time of the noise exposure (Table I) All the details of the noise-exposure technique were as previously described (Kiang, Liberman & Levine 1976) Seven of the routine animals were exposed to narrow-band noise with center frequency at either

reotactic radiosurgery in the treatment of acoustic neurinomas The experience gained in this first study indicate that radiosurgery offers an alternative to traditional surgical methods in cases of small and medium sized acoustic tumours A great advantage is that the risk of severe damage to facial nerve and impaired hearing function is minimized the latter an important fact when approaching such difficult problems as bilateral tumours The non invasive radiosurgical procedure and the very short and uneventful postoperative course are factors that would make it possible to perform this treatment on an outpatient basis

ZUSAMMENFASSUNG

Von 1969 bis 1974 wurden 9 Patienten mit Akustikusneurinomen mit stereotaktischer Radiochirurgie behandelt und die Nachbeobachtungszeit kann jetzt für eine vorläufige Beurteilung des Wertes dieser Behandlungsmethode als ausreichend angesehen werden. In 8 von den 9 Fällen wurde Wachstumsstillstand oder Verkleinerung des Tumors beobachtet. In einem Fall wurde 2 Jahre nach der Bestrahlung der Tumor herausoperiert, und man fand bei der histologischen Untersuchung des Präparats regressive Veränderungen des Typs wie er nach Strahlenbehandlung zu erwarten ist. Audiologische Untersuchungen ergaben daß der radiochirurgische Eingriff in der Mehrzahl der Fälle zu keiner wesentlichen Verschlechterung des Hörvermögens geführt hat. In den erfolgreichsten 7 Fällen wurde die präoperative Hörminderung nur um durchschnittlich 20 0 dB verstärkt. *In keinem Fall kam es zu einer Schädigung des N facialis* Dieses Verfahren stellt eine befriedigende Alternative in der Behandlung von kleinen oder mittelgroßen Akustikustumoren dar die wert ist, in Betracht gezogen zu werden.

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METHODS

The animals used in this study included 6 cats born and raised in a low-noise chamber (Liberman 1978) and 9 routine cats (obtained from local animal suppliers) exposed to acoustic trauma. All of the routine animals weighed *less than 2 kg* at the time of the noise exposure (Table I) All the details of the noise-exposure technique were as previously described (Kiang, Liberman & Levine 1976) Seven of the routine animals were exposed to narrow-band noise with center frequency at either

Table I *Information on traumatized animals, including weight at time of noise exposure, center frequency, bandwidth and intensity (at tragus) of traumatizing stimulus, duration of exposure, and survival time between exposure and auditory-nerve experiment*

Cat no	Weight (kg)	Center Frequency (kHz)	Bandwidth (Hz)	Intensity (dB SPL)	Duration (hours)	Survival Time (days)
101	1.0	3.0	50	111	2	487
104	0.7	0.75	50	112	2	509
106	1.8	6.0	200	116	2	693
108	1.3	3.0	4 500	108	2	637
110	0.5	1.5	50	111	2	570
111	1.8	3.0	4 500	115	2	620
112	0.4	1.5	50	110		598
114	0.7	0.75	50	111		713
116	1.7	5.5	1 000	115	2	125

0.75, 1.5, 3 or 6 kHz. Two other routine animals were exposed to a two-octave band of noise with cutoff frequencies at 1.5 and 6 kHz. The auditory-nerve experiment was carried out from 125 to 713 days after the noise exposure (Table I). The chamber raised animals were from 3 to 6 months old at the time of sacrifice.

The techniques involved in exposing the auditory nerve, recording from single auditory nerve fibers, presenting and calibrating the acoustic stimuli, and processing responses have been described in previous publications (Kiang et al., 1965; Liberman, 1978). Electric shocks were used in all experiments as search stimuli while advancing the microelectrode (Liberman, 1978).

Fixation and tissue preparation

After the auditory nerve experiment, the cochleas were prepared for histological examination as epon-embedded surface preparations (Bohne, 1972; Spoendlin & Brun, 1974). Cochleas were fixed by perfusion with glutaraldehyde in a phosphate buffer at pH 7.3, postfixed with osmium tetroxide in phosphate buffer, dehydrated, and embedded in epon. After hardening, the superficial epon and petrous bone were drilled away, leaving an epon cast of the cochlear scalae containing the intact organ of Corti. By a series of razor cuts the entire length of the organ of Corti could be divided into approximately 30 pieces without losing any hair cells. For each piece, excess epon above and below the organ of Corti was ground away until all the hair cells could be examined with a 100× oil-immersion objective. The resolution increased steadily as the piece was thinned.

Cytocochleograms

Because some of the hair cell loss described in the results is minimal, the exact way in which the cytocochleograms were derived is of some importance. In each piece of the cochlea, the IHCs were numbered consecutively from the basal to the apical end of the piece. The locations of any missing IHCs were noted by recording the appropriate position number. The notation of outer hair cell (OHC) loss was complicated by the scattered irregularities of the OHC pattern in the reticular lamina. Hair cell loss marked by phalangeal scars (Engström, Ades & Andersson, 1966) is easy to interpret. In some cases, however, there are no scars in regions where less than a full complement of OHCs is present. Although this irregularity is more common in the apical regions, it can be seen throughout the cochlea. For the cytocochleograms in this paper, these irregularities as well as those marked by scars are treated as "miss

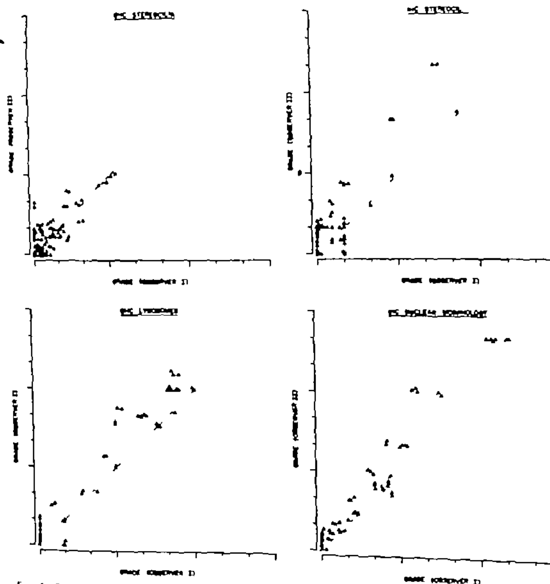


Fig. 1. Comparison of the evaluation of four aspects of hair cell conditions as performed by two observers. The dotted lines indicate where all points would fall if there were perfect agreement between the observers. Each point represents the grade given by each observer as averaged over 5% of the length of a given cochlea.

ing hair cells (unless otherwise specified). Supernumerary OHCs are ignored.

Cochlear lengths were determined by tracing the union of the pillar heads at a magnification of 100×. The average length for an entire cochlea was 24.5 mm. Cochlear lengths within individual pieces were estimated by interpolation based on the location number of the particular hair cells in question. For the final cytocochleograms, the total length of the cochlea was divided into 100 bins (linearly spaced). Each bin contained approximately 26 IHC and 32 OHC locations from each row.

Hair cell condition analysis

The analysis of hair cell condition was performed by two observers. During this analysis neither observer had knowledge of the physio

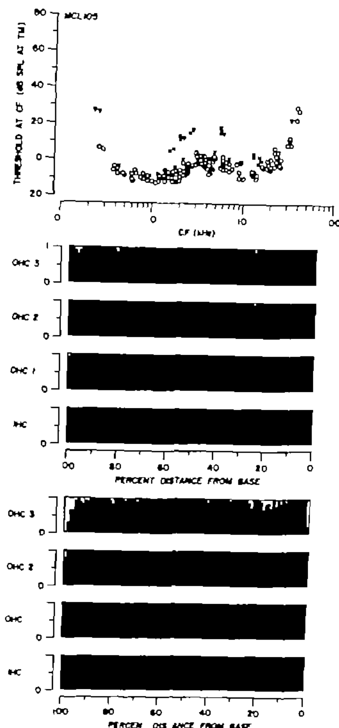


Fig 2 Comparison of single-unit thresholds and two types of cytocochleograms for the standard cat Each point in uppermost panel represents the threshold at the CF of a different unit. The threshold is given in dB re 0 0002 dynes pe square centimeter as measured near the tympanic membrane (*TM*) I this and all subsequent figures O *represent units with SRs greater than 18 spikes per second* ▼ represent units with SRs less than 0.5 spikes per second × *represent units w th intermediate SRs* The cytocochleogram plot with a bin width of 1% of cochlear length the fraction of hair cells remaining (in black) In the upper plot a hair cell is considered to be missing only if there a phalangeal scar in the lower one scarless hair cell absence is also included (see Methods)

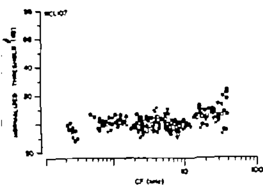
logical data associated with the cochlear slides because all identifying marks on the slides were obscured and the slides from all cochleas were intermixed The slides were divided into 20 groups according to cochlear location The task was to compare at one sitting all the slides from one group and grade them on a series of histological features. The features evaluated were (1) the orderliness of the stereocilia for each of the four rows of hair cells (2) the quantity of subcuticular osmiophilic bodies (lysosomes) (Engström & Ades 1960 Lim & Melnick 1971) within the OHC cytoplasm (one grade for all three rows) or within the cytoplasm of the IHCs and their associated supporting cells (3) the degree of pyknosis of the IHC cytoplasm (4) the degree of crenation of the IHC nuclei and (5) the amount of crenation and/or swelling of the OHC cytoplasm For each of these five features the observers assigned grades between A and D where A" and D were meant to represent the best" and worst" cases, respectively The extremes between which each feature could vary were identified in a preliminary analysis of the histological material The agreement between the two independent sets of observations can be seen in Fig 1

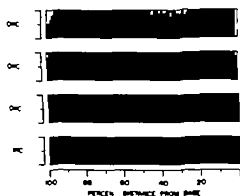
RESULTS

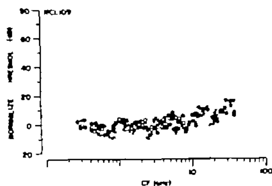
A Correlating physiopathology and patterns of hair cell loss

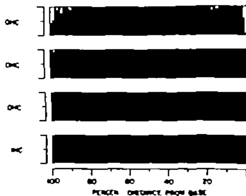
For this study the auditory-nerve response from animals born and raised in a low-noise

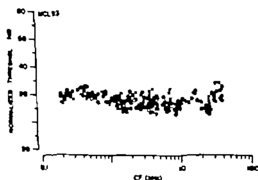
Fig 3 Comparison of normalized threshold plots and cytocochleograms for 4 of the chamber-raised animal The normalized threshold is defined as the difference between a given unit s threshold t CF and the average threshold at CF for the units of similar CF and the same SR group from the standard cat (MCL 105) In this and all subsequent figures a vertical arrow associated with filled triangle indicates that a low SR unit was isolated w th a weak response to a tone at the highest levels routinely presented The cytocochleogram In this and all subsequent figures are made according to the criteria described for the low r cytocochleogram in F

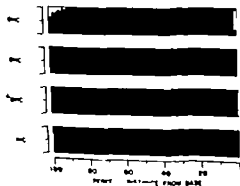

10
100

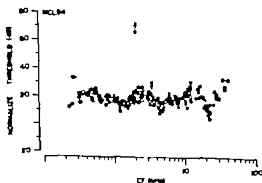

OHC
OHC
OHC
IHC
100
80
60
40
20

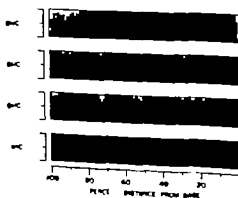

80
60
40
20
0
20
10
100


OHC
OHC
OHC
IHC
100
80
60
40
20


10
100




80
60
40
20
10
100


100
80
60
40
20

environment will serve as a physiological standard against which data from noise exposed (NE) ears will be compared Single units recorded from chamber raised (CR) animals show exceptionally low thresholds at the characteristic frequency (CF) The threshold distribution obtained in the most sensitive of these animals is shown in Fig 2 In this particular case minimum thresholds at all CF regions were 10 to 15 dB lower than ever seen in routine animals Note the significant differences in threshold for units with different spontaneous discharge rates (SRs) In all subsequent plots of threshold at CF the data will be normalized with respect to the distribution seen in MCL 105 (Fig 2) hereinafter termed the standard cat Thresholds for units from each of the three SR groups (Liberman 1978) will be normalized with respect to the average threshold for units from the same SR group (and the same CF region) from this standard cat

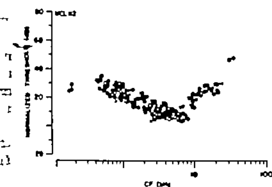
There was very little hair cell loss seen in the cochlea from the standard animal (Fig 2) There were no missing IHCs and only seven of the 3015 first row OHCs had been replaced by scars The great majority of deviations from the "ideal" hair cell array occurred in the form of scarless absence mainly in the third row OHCs (lower cytocochleogram) These deviations may represent congenital absence rather than acquired losses

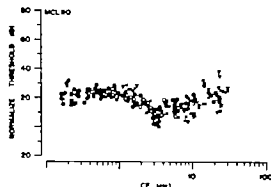
The cytocochleograms and normalized threshold plots for four other CR animals are shown in Fig 3 For two of the cases MCL 107 and 109 the normalized thresholds at all CF regions and for all SR groups are near zero indicating that the threshold distributions were almost identical with that of the standard cat The cytocochleograms are also similar to the standard almost no missing cells anywhere except among the third-row OHCs In the other two cases MCL 93 and 94 the thresholds were higher than the standard However only for MCL 94 was there a more than standard amount of hair cell loss especially throughout the first and second row of OHCs The degree of hair cell loss in this case cannot be correlated with a significant threshold shift for the high- or medium-SR units (compare MCL 93 and 94) It may however be correlated with the exceptionally high thresholds of the low SR units. In the data from MCL 94 the low SR units showed thresholds up to 70 dB higher than their counterparts in the standard animal Thus it appears that the thresholds of the low SR units have been shifted to a greater degree than the high or medium-SR units resulting in a greater threshold spread at any one region of CF

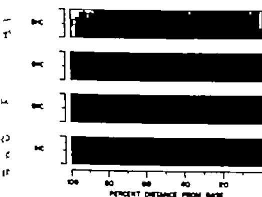
All of the cases exposed to acoustic trauma showed an apparently larger threshold shift for the low-SR units than for the high-SR units of similar CF Cochleograms and normalized threshold plots for four of these cases are shown in Fig 4 The threshold "shift" for the high SR units in these cases is less than 40 dB except for CFs greater than 20 kHz Similar threshold shift can be seen among the less sensitive of routine-normal animals (Liberman & Kiang 1978) Thus the noise-induced lesions if any are minimal in these four cases In each case the low-SR units showed threshold shift of greater than 50 dB in some CF region Correspondingly each of the cytocochleograms showed significantly more scattered OHC loss in the first and second rows than any of the cochleas from CR cats (except MCL 94) These data suggest that threshold shift in the low SR units might be a sensitive indicator of minimal cochlear damage

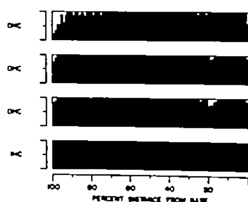
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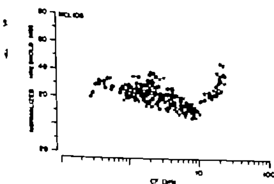
Fig 4 Comparison of cytocochleograms and normalized threshold plots for 4 noise-exposed cats showing minimal lesions The crosshatching of the cytocochleograms for the apical regions of two cochleas is to signify some uncertainty as to the precise pattern of hair cell loss. Technical difficulties in these pieces of the cochlea made it very difficult to assign outer hair cells to the different rows.

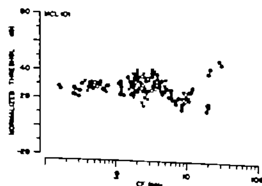

MCL 102
NORMALIZED THRESHOLD (dB)
CF (kHz)

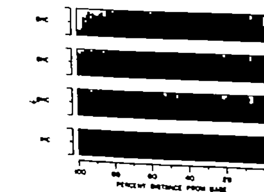

MCL 110
NORMALIZED THRESHOLD (dB)
CF (kHz)

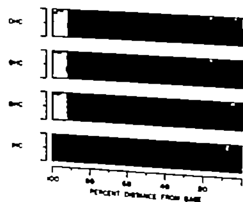

OHC
OHC
OHC
IHC
PERCENT DISTANCE FROM BASE


OHC
OHC
OHC
IHC
PERCENT DISTANCE FROM BASE


MCL 108
NORMALIZED THRESHOLD (dB)
CF (kHz)


MCL 101
NORMALIZED THRESHOLD (dB)
CF (kHz)


OHC
OHC
OHC
IHC
PERCENT DISTANCE FROM BASE


OHC
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Fig 4 Comparison of cytocochleograms and normalized threshold plots for 4 noise-exposed cats showing minimal lesions The crosshatching of the cytocochleograms for the apical region of two cochleas is to signify some un certainty as to the precise pattern of hair cell loss Tech nical difficulties in these pieces of the cochlea made it very difficult to assign outer hair cells to the different rows.

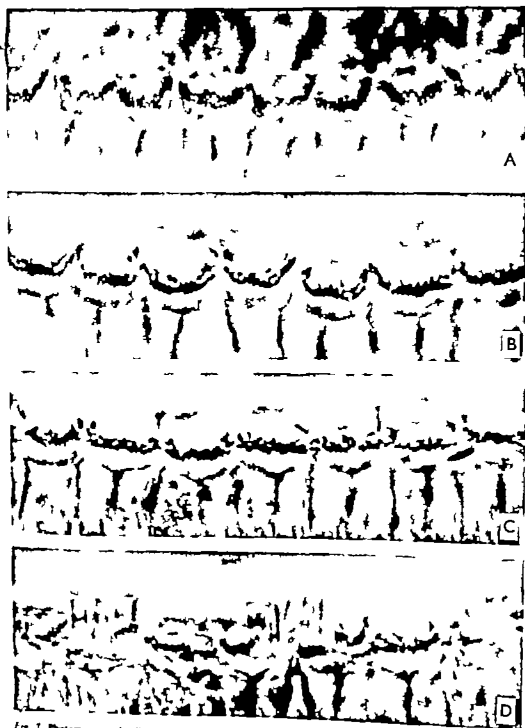


Fig. 7. Photomicrographs illustrating different degrees of disorderliness of the IHC stereocilia. The letter indicates the grade assigned to the stereocilia in each region. The top micrograph (*A*) was taken from MCL93 (one of the chamber-raised animals) in the cochlear region 70% of the distance from the base. Panel *B* shows stereocilia from MCL101 from the cochlear region 66% of the way from base to apex. The micrographs labeled *C* and *D* were taken from MCL104 in the cochlear regions 74% and 86% of the distance from the base, respectively.

Fig 5 Comparison of normalized threshold plots and cytocochleograms for noise-exposed animals All conventions for data display are as described in the captions to Figs 3 and 4

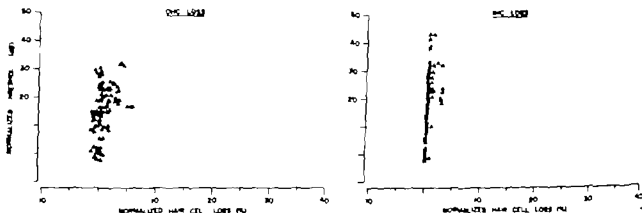


Fig 6 Correlation between the amount of hair cell loss and the average threshold shift for all cochlear regions from all cats in the study The percentage loss of outer and inner hair cells (horizontal axes) is averaged over 5% increments of cochlear length To arrive at the "threshold shift" associated with any given 5% length of the cochlea from a given case the appropriate CF boundaries are determined and the average difference in threshold at CF is determined between the high-SR units from that case and the average threshold at CF for high-SR units of similar CF from the standard cat (MCL 105).

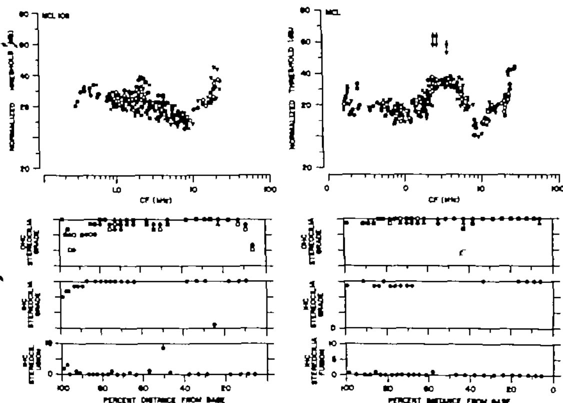


Fig. 9. Comparison of normalized threshold plot and the condition of the stereocilia for 2 noise-exposed ears. All conventions for data display are as described in the caption to Fig. 8.

regions of stereocilia according to how closely they approached either extreme.

The ratings given to all the stereocilia from of the CR cats are shown in Fig. 8. In the great majority of the slides from these cochleas, the IHC and OHC stereocilia were given the highest ratings ("A" or "A") for orderliness.

In each of the NE animals, there was at least one cochlear region in which the IHC or OHC stereocilia appeared significantly more disorderly than in the cochleas of CR animals. The pattern of stereocilia abnormalities in two NE ears is shown in Fig. 9. Note, especially for MCL 111, that the cochlear region showing the greatest disorderliness of the stereocilia was associated with the CF region showing maximal threshold shift. In that region, the worst damage seemed to have been to the IHCs and first row OHCs, a pattern which has been reported in other studies of acoustic trauma (Soudijn, 1976). For MCL 108, the extreme disorderliness of the IHC stereocilia in the cochlear region 20–30% of the distance from the base is probably artifactual. Evidence for this interpretation will be presented below.

A summary of the correlation between threshold at CF and the condition of the stereocilia is shown in Fig. 10. Damage to the stereocilia from either hair cell group was correlated with threshold shift, but the best correlation resulted by combining the degree of damage to the OHC and IHC stereocilia in each region of the cochlea (as has been done in the figure). For this scatterplot, data from the apical 15% or the basal 30% of the cochlea have been excluded. In these regions, there

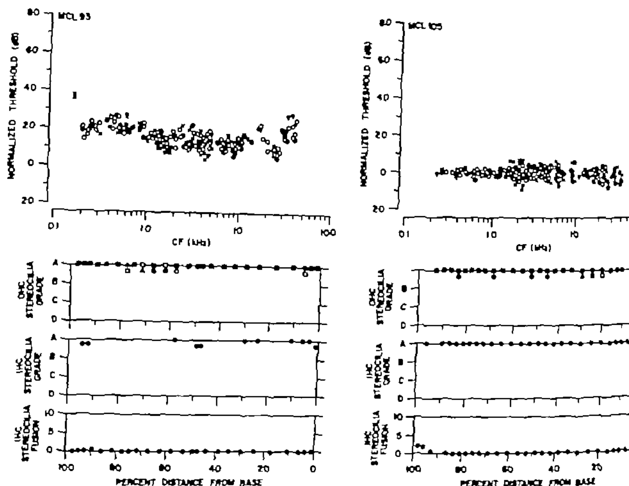


Fig 8 Comparison of normalized threshold plots and condition of stereocilia in data from 2 chamber-raised cats In the lower three panels of each column squares represent OHC1 triangles OHC circles, OHC3 and filled circles IHC The vertical axis for the bottom p of each column is an estimate of the percentage of IHC stereocilia which were fused

which the average OHC loss exceeds 20% (*in any 1% increment of cochlear length*) are shown in Fig 5 Both cases show a focal lesion of OHCs especially severe for the first row In the CF regions correlated with these losses the high SR thresholds are shifted by more than 40 dB The correlation between OHC loss and threshold shift among the high and medium SR units is shown more explicitly in Fig 6 Note that in many cases there is significant threshold shift which cannot be associated with hair cell loss even after more than one year survival from acoustic trauma

B *Correlating physiopathology and patterns of hair cell damage*

There were clear differences in the condition of the hair cells which remained in the cochleas of NE and CR animals One cytolog difference which is easy to see but difficult describe is the orderliness of the stereocil The top panel of Fig 7 shows some IH stereocilia from one of the CR animals Ea ciliary tuft shows a very regular array stereocilia Contrast this micrograph with th in the bottom panel In the latter case ma of the stereocilia have been bent near the roots so that they lie flat against the cuticul plate Those tufts that remain erect appe "ruffled" when compared with those from th CR ear The stereocilia shown in the top ar bottom micrographs illustrate the most an least orderly of the stereocilia seen in the case from this study These extremes were give ratings of "A" and D" respectively and th task of the observers was to rate all othe

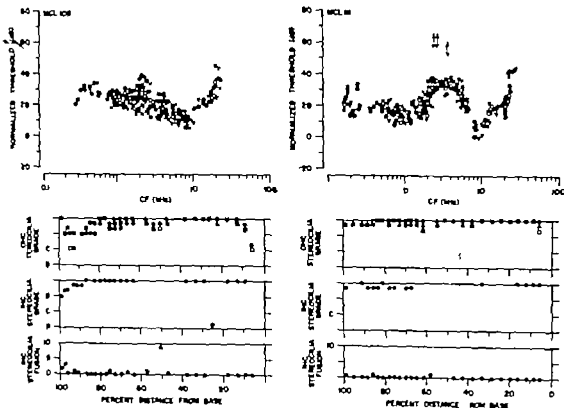


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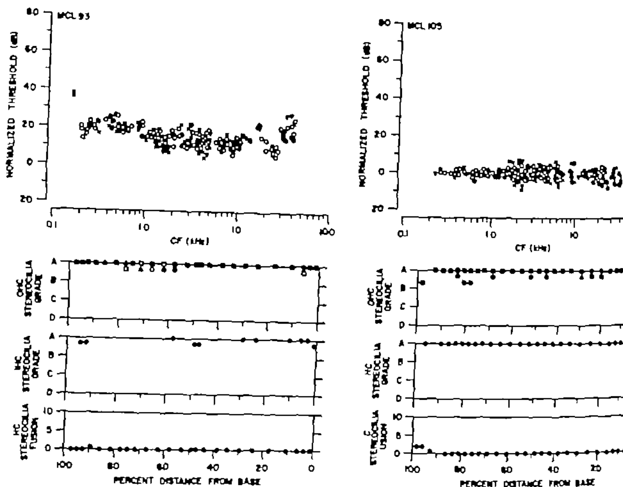


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Fig. 11. Correlation between average threshold shift and estimates of lysosome density associated with OHCs or IHCs. Average threshold shift is derived as described in the caption to Fig. 6. The average grad for lysosome activity for either IHC or OHCs is averaged across given 5% increment of cochlear length and averaged across both observers as well. Filled triangles represent data from chamber raised animals.

attributed to errors in determining sound pressure at the tympanic membrane at high frequencies and perhaps to inaccuracies in the cochlea map (outside of the 0.4 to 14 kHz range the map is based solely on extrapolation). Another possible reason for the weak correlation in the basal and apical extremes is that the histological picture there can be confused by artifactual changes in stereocilia condition. During the evaluation of hair cell condition both observers noted the occurrence of what they considered to be gross mechanical artifact in the histological material. Identifiable artifacts were limited to the apical 15% and basal 30% of the cochlea. Every piece in which a mechanical artifact was noted also showed significant disorderliness of the stereocilia. For example, the cochlea from MCL 108 (Fig. 9) showed a clear mechanical artifact at a location roughly 25% of the distance from the base. In this case the artifact was seen as a clear break in the organ of Corti which probably occurred immediately after fixation. There was a blood clot on the underside of the basilar membrane and an attempt had been made to remove it with a fine probe. Disruption of the stereocilia extended for 1.4 mm toward the base from the point of basilar-membrane rupture (and not at all apical to it). Similar abrupt changes in the orderliness of stereocilia were associated with each case of mechanical artifact. Thus, it appears that mechanical disruption of the organ of Corti during tissue

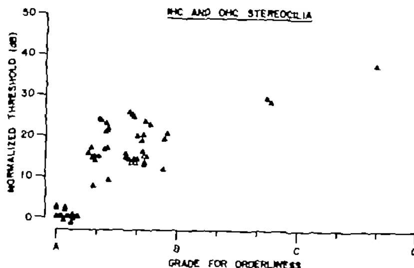


Fig 10 Correlation between disorderliness of the stereocilia on OHCs and IHCs and the threshold shift seen in high-SR units. Data are included from all animals in this study. The abscissa is derived as described in the caption to Fig. 6. The ordinate is derived by averaging over each 5% of cochlear length for each case the grade assigned by the two observers for the orderliness of the stereocilia on all four rows of sensory cells.

were several cases in which significant disorderliness of the stereocilia could not be associated with significant threshold shifts. Possible reasons for this discrepancy will be discussed below.

Fusion of the stereocilia was more common in the NE ears than in the CR ears. In the latter, fusion was completely confined to the IHCs in the apical 30% of the cochlea where in each case a few scattered IHCs might show fusion of less than 1/3 of their cilia. Among the NE ears, fusion was more severe in that (1) it was seen throughout the upper 50% of the cochlea, (2) it was seen on more IHCs in each case, and (3) on any given cell, all of the stereocilia could be fused. The location and extent of this fusion, however, was not closely correlated with the degree of threshold shift. MCL 108 (Fig 9) showed some of the most extensive fusion of all the cases, but the threshold shift in the associated CF region was only moderate, and there was no threshold notch associated with the region showing the worst fusion. This lack of correlation is not surprising in view of the fact that, even in the worst region of this worst case, less than 10% of the stereocilia were involved.

The density of subcuticular lysosomes was also significantly lower in the hair cells from CR animals than in data from NE animals (Fig 11). This resulted in a weak correlation across all animals between threshold and lysosome density. However, among the NE ears there was no correlation (Fig 11) and in any one animal, notches in the threshold distribution were not associated with peaks in the lysosome density. It may be that the differences in lysosome density among our animals are correlated with age rather than noise exposure. The CR animals were all less than 6 months old, while the animals with chronic acoustic trauma were all at least 2 years old. When a young CR animal was exposed to acoustic trauma and sacrificed 7 months later, there was no obvious increase in lysosome density in the hair cells from the damaged region (Liberman, unpublished observation).

DISCUSSION

A. *Artifacts and the assessment of hair cell condition*

Of all the indices of hair cell damage we examined in the light microscope, the only one which was strongly correlated with threshold shift was the orderliness of the stereocilia. This correlation, however, was strong only if one excluded data from the apical 15% and the basal 30% of the length of the cochlea. According to our cochlear length–frequency map, the excluded CF regions would be those below 0.4 kHz or above 15 kHz. The lack of correlation for CF above 15 kHz could be

In general the longitudinal and radial distribution of the worst stereocilia damage is complementary to the longitudinal and radial distribution of the "imprints" of the stereocilia in the underside of the tectorial membrane. That is to say fusion is most common on the apical IHCs and only for apical IHCs has it been difficult to demonstrate that the sensory hairs insert into the substance of the tectorial membrane (Kimura 1966 Hoshino 1976). Perhaps the insertion of the stereocilia into the tectorial membrane reduces the opportunities for contact between adjacent stereocilia, thus reducing the opportunity for noise induced fusion and disorderliness. This type of speculation could be extended to explain the observation that among the OHCs the stereocilia on the first row cells are most vulnerable to disorderliness (Soudjin 1976 Hunter Duvar 1977). The OHC I stereocilia are significantly shorter than those on the other two rows and thus may be less firmly anchored in the tectorial membrane.

C *Contribution of hair cell populations to single-unit thresholds*

In our data the selective loss of OHCs appears to be correlated with elevation of thresholds at CF (Figs 5 and 6). Similar results have been reported in other studies with less restricted lesions (Ryan & Dallos 1975 Evans & Harrison 1975 Dallos & Harris, 1978). In our cases of chronic acoustic trauma, most of the threshold shift could be accounted for by structural changes in the organ of Corti which were clearly visible in the light microscope (Figs. 6 and 10). Although threshold shift was not always associated with hair cell loss in most cases it could be correlated with damage to either OHC or IHC stereocilia. It may be that disruption of the stereocilia on either hair cell group alters events at the micromechanical level thereby elevating the thresholds at CF.

Minimal cochlear damage seems to raise the thresholds of low SR units more than that of the high or medium classes thus increasing the threshold spread in each region of CF (Figs 3 and 4). In the data from the most sensitive of the chamber-raised animals the low SR units were about 30–40 dB less sensitive than the high SR units and 10–20 dB less sensitive than the medium-SR units. In all noise-exposed animals in most routine normal animals and even in one of the chamber raised animals, the low SR units were as much as 70 dB less sensitive than high SR units of similar CF. In many cases this increased threshold spread could be correlated with scattered OHC loss (Figs 3 and 4).

The suggestion that low SR units might be sensitive indicators of low levels of cochlear damage could be useful in interpreting physiological data from the auditory nerve. The pattern of thresholds for high-SR units allows certain predictions to be made about the pattern of hair cell loss in the cochlea (Liberman & Kiang 1978). However when comparing the high-SR thresholds for two different animals it is always possible that differences in transmission characteristics of earlier stages such as the middle ear could add threshold differences (on the order of 20–30 dB) which might obscure the correlations with cochlear histopathology. Differences between ears in amount of threshold *spread* however cannot be attributed to conductive lesions.

The possibility that the low SR units are more easily damaged than high- or medium-SR units strengthens the overall impression that this small subset of auditory-nerve units is quite different from all other units recorded from the nerve bundle (Liberman 1978). The morphological significance of this distinction is not yet clear.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of E. M. Marr K. E. Hackel and O. S. Roberts. Thanks are also due to N. Y. S. Kiang and T. F. Weiss for helpful criticism of the work and the manuscript. This work was supported by US Public Health Service Grant 5 P01 NS13126.

processing typically causes disorderliness of the hair cell stereocilia

If this conclusion is true it has serious consequences for the study of stereocilia condition via the scanning electron microscope (SEM) Preparation of the cochlea for the SEM involves much more handling of the cochlea than our preparation since for the SEM the bone of the cochlea must be drilled away extensively the organ of Corti is often cut and adjacent tissue of the cochlear duct is removed In some SEM studies on stereocilia condition including pioneering efforts (Engström Ades & Bredberg 1970) and more recent work (Soudjin 1976) the hair cells (especially IHCs) which are described as normal" would have been rated as significantly abnormal by our criteria Moreover the disorderliness of stereocilia that we associated with mechanical artifact can be similar in degree to that described in SEM studies as noise-induced damage (Hunter Duvar 1977)

B *Cellular reactions to acoustic trauma*

The noise-exposed animals in this study had times as long as 2 years Since these are longer than in most other studies it is of some interest to compare results with those for shorter survivals

When survival times are from one to three months there can routinely be up to 50 dB of noise-induced threshold shift when there is only minimal hair cell loss in the appropriate region of the cochlea (Liberman & Kiang 1978) Among the long term survival cats in the present study there were three for which the threshold shift exceeded 40 dB In each of these cases there were focal regions of significant hair cell loss in that part of the cochlea associated with the CF regions showing threshold shifts (Figs 5 and 6) If these 3 cases are taken to represent typical patterns for acoustic trauma of long-standing then there is a significant difference in the relation between hair cell loss and threshold shift at 2 month survival and at 1½ year survival If the threshold shifts have stabilized by 2 months after the trauma then some of hair cells which were present (but dysfunctional) at survival times of a few months have by survival times of 1½ years been eliminated Current suggestions for cellular mechanisms underlying the noise-induced loss of hair cells (Bohne 1976) seem inadequate to explain a loss of hair cells which might occur many months after the insult

There is another difference in the correlations found between histopathology and pathophysiology at short and long survivals. When data were analyzed from animals with survival times of a few months there were many cases in which significant noise induced threshold shift could not be correlated with either hair cell loss or stereocilia damage (Liberman & Kiang 1978) whereas in the present study almost all threshold shift could be correlated with one or the other measure This discrepancy does not have to be attributed to the difference in survival times, since a different histological technique (celloidin sections) was used in the previous study Although we evaluated the stereocilia in the sectioned material it was clear at the time that different degrees of orderliness were difficult to discriminate with that technique Furthermore we have recently seen correlation between disorderliness of the stereocilia and threshold shift in ears with minimal hair cell loss at survival times as short as 2 months, using the surface preparation technique (Liberman unpublished observation)

In both the short and long-term material we have examined fusion of stereocilia was confined almost exclusively to the IHCs, in the apical half of the cochlea Although fusion was seen in the data from chamber-raised animals it was significantly more common in most of the noise-exposed ears In cochleas with large regions of hair cell loss the region bordering the loss typically show isolated IHCs with severely fused and/or very disorderly stereocilia whereas isolated OHCs can have stereocilia that look essentially normal (Liberman unpublished observation)

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ZUSAMMENFASSUNG

Es werden Ergebnisse einer physiologischen und histologischen Studie gezeigt welche einesteils an speziell lärmgeschützt aufgewachsenen Katzen unternommen wurden anderenteils an Katzen welche vorgängig akustisch traumatisiert worden waren Die bis anderthalb Jahre nach der Traumatisierung beobachteten Katzen hatten in den elektrophysiologisch abgeleiteten Einzelnervenfaserpotentialen bis zu −40 dB erhöhte Hörschwellen was zeitweise nicht mit einem signifikanten Haarzellenverlust inherging (Beobachtungen an in Epon eingebetteten Oberflächenpräparaten) Unter all den untersuchten histologischen Merkmalen war es eine regellose Anordnung der Stereocillen in den äußern und innern Haarzellen welche am besten mit den Nervenfaserhörschwellen korreliert waren Zum Schluß zeigte sich daß die meisten Hörschwellenänderungen in den lärmexponierten Cochleas entweder durch einen Verlust oder durch eine Schädigung der Haarzellen — beide Parameter deutlich sichtbar unter dem Lichtmikroskop — erklärt werden konnten

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UPTAKE OF PUTATIVE NEUROTRANSMITTERS IN THE ORGAN OF CORTI

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Abstract In vitro uptake of putative neurotransmitters into the organ of Corti of the guinea pig was studied by autoradiography After incubation in ^{3}H-glycine the label was heaviest over the inner hair cell, but was not confined to the synaptic region of the cell. After incubation in ^{3}H-GABA, ^{3}H-glutamate and ^{3}H-aspartate heavy labeling was seen over the fibers and terminals of the efferent olivocochlear bundle Leucine an amino acid not thought to be a neurotransmitter was uniformly taken up by all cochlear structures The fact that GABA glutamate and aspartate are taken up into efferents, which are almost certainly cholinergic suggests that high affinity uptake of these substances is not restricted to terminals in which these substances are released as neurotransmitters

Most putative neurotransmitters are taken up into neuronal and glial elements through a high-affinity sodium-dependent process The physiological role of this process has not been determined but it is believed to be active in the removal of neurotransmitters from the synaptic cleft Some putative neurotransmitters, for example catecholamines are specifically taken-up into presynaptic terminals from which they are released (Molinoff & Axelrod 1971 Iversen, 1974 Iversen et al 1975) Using radioactive substances and autoradiography this uptake process provides a method for identifying synapses which may use a specific catecholamine neurotransmitter (Molinoff & Axelrod, 1971 Iversen & Schon 1973 Schon & Iversen 1974) Uptake has also been used to localize putative amino acid synapses (Schon & Iversen 1974 Iversen 1974 Iversen et al. 1975) although evidence for an uptake of these substances into specific presynaptic terminals is less compelling.

The mammalian cochlea has synapses between hair cells and afferent fibers and synapses between efferent fibers and hair cells or afferent fibers Evidence suggests ACh may be the major transmitter for the efferent system (Fex 1974 Fex & Wenthold 1976 Godfrey et al 1976 Guth et al 1976 Fex & Adams 1978) However little information is available on the neurotransmitter for the hair cells In the present study we have mapped autoradiographically the distribution of silver grains over the organ of Corti incubated in vitro in solutions containing different putative neurotransmitters In vitro conditions were used to control better the concentration and availability of the substances tested It was intended that these studies might help to characterize the hair cell neurotransmitter

METHODS AND MATERIALS

NIH guinea pigs weighing between 250 and 325 g were decapitated the left temporal bone was taken out and the bulla was opened and chipped away as was most of the bone around the cochlea. The cochlea was dissected in a phosphate-buffered saline solution containing 170 mM NaCl 5 mM KCl 10 mM glucose 1.3 mM MgSO and 20 mM NaPO at pH 7 4 bubbled with 95% O_2+5% CO_2 The cochlear shell and part of the spiral ligament with the stria vascularis was removed The bony modiolus with the organ of Corti from the end of the first turn to apex, was transferred under

ZUSAMMENFASSUNG

Es werden Ergebnisse einer physiologischen und histologischen Studie gezeigt welche einesteils an speziell lärmgeschützt aufgewachsenen Katzen unternommen wurden, anderenteils an Katzen, welche vorgängig akustisch *traumatisiert* worden waren Die bis anderthalb Jahre nach der Traumatisierung beobachteten Katzen hatten in den elektrophysiologisch abgeleiteten Einzelnervenfaserpotentialen bis zu −40 dB *erhöhte Hörschwellen* was zeitweise nicht mit einem signifikanten Haarzellenverlust einherging (Beobachtungen an in Epon eingebetteten Oberflächenpräparaten) Unter all den untersuchten histologischen Merkmalen war es eine regellose Anordnung der Stereocilien in den äußern und innern Haarzellen welche am besten mit den Nervenfaserhörschwellen korreliert waren Zum Schluß zeigte sich daß die meisten Hörschwellenänderungen in den lärmexponierten Cochleas entweder durch einen Verlust oder durch eine Schädigung der Haarzellen — beide Parameter deutlich sichtbar unter dem Lichtmikroskop — erklärt werden konnten

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Table I *Distribution of label over the organ of Corti after incubation with tritiated putative neurotransmitters*

	H_3-GABA	H_3-Glutamat	H_3-Aspartate	H_4-Glycine
Neural structures				
Inner hair cells	−	−	−	+
Outer hair cells	−	−	−	−
Inner spiral bundle	++	++	++	−
Tunnel spiral bundle	++	++	++	−
Tunnel crossing fibers	+	+	−	−
Base of outer hair cells		+	−	−
Nerve fiber region of habenula		−	−	−
Non-neural structures				
Stria vascularis	−		+	++
Basilar membrane		+	++	−
Reissner membrane		++	+	++
Habenula	+	−		−
Schwann cells	+		−	−

a *The amount of label over each cochlear structure is xpressed relative to background for that amino acid*

hair cells (Fig. 5) These grains are evenly distributed *over the entire cell including over* the region of the cell which contributes to the reticular lamina The efferent and afferent fiber bundles are unlabeled as are the outer hair cells

Leucine

After incubation in ³H-leucine there is a uniform *distribution of* label over *all* structures of the organ of Corti modiolus stria vascularis and Reissner s membrane (Fig 6) The label does *not* accumulate preferentially over any *region or* structure in the organ of Corti

DISCUSSION

Glutamic acid and aspartic acid are generally considered to be excitatory neurotransmitters based on their *high concentrations in neuronal* tissue and their neuronal excitatory effects They have been suggested as the *afferent* transmitters in the acousticolateralis system (Steinbach & Bennett 1971 Steinbach 1974 Teeter & Bennett 1975) but the evidence is weak and other studies *have shown that* glutamic acid and aspartic acid are not potent excitants in the cochlea (Klinke & Oertel 1977 Bobbin & Thompson, 1978) Glycine and GABA are considered to be inhibitory putative *neurotransmitters GABA has been suggested* as the hair cell neurotransmitter based on its synthesis and the uptake of its precursor in the basilar papilla and lateral line organ (Flock & Lam *1974) However* studies have shown a low concentration of GABA and its synthesizing enzyme in the organ of Corti (Tachibana & Kuriyama 1974 Fex & Wenthold 1976 Godfrey et al 1976) indicating it is unlikely that GABA serves as a major transmitter in any capacity in the cochlea.

Our results show that all substances studied are accumulated by cochlear structures Leucine not considered a neurotransmitter is heavily taken up and uniformly distributed throughout the cochlea probably reflecting protein synthesis Glycine is concentrated in inner hair cells but not specifically in the synaptic region

In the present study glutamic acid aspartic acid *and GABA* appear to be associated with efferent fibers and terminals In the guinea pig, after entering the organ of Corti through the habenula *efferent* fibers from the olivocochlear bundle travel within the inner spiral bundle and tunnel spiral bundle (Wright & Preston, 1973 Wright 1975 Wright & Preston, 1975) Within the inner spiral bundle

fluid to a conical Beem capsule for incubation and further processing

The bony modiolus and organ of Corti were incubated for 20 minutes at 30°C in 100 μl of the buffer with the radioactive substance at a concentration of 10^{-6} M The ^{3}H amino acids used for incubation were obtained from New England Nuclear Boston Massachusetts at the following specific activities GABA 36 7 Ci/mmole glutamic acid 23 4 Ci/mmole as partic acid 17 8 Ci/mmole glycine 9 4 Ci/mmole leucine 50 0 Ci/mmole After a brief wash in the buffered saline the tissue was fixed for 1 hour in 4% paraformaldehyde in 0 1 M sodium cacodylate with 20 mM $CaCl_2$ The spiral was dissected into turns washed in 0 2 M sodium cacodylate with 20 mM $CaCl_2$ and postfixed in 2% OsO_4 in 0 1 M sodium cacodylate The tissue was then dehydrated in methanol and embedded in Araldite From each turn 30 sections 1–2 μm thick were cut and placed on slides The slides were dipped in NTB 2 Kodak emulsion and stored at 4°C for 4 weeks before developing in D-19 The sections were stained with methylene blue–Azure II

RESULTS

The distribution of the ^{3}H amino acids over the various cells and regions of the organ of Corti is summarized in Table I

GABA

In vitro incubation of the organ of Corti in ^{3}H-GABA yields a pattern of labeling like that seen after in vivo incubation (Richrath et al 1974) There is a heavy accumulation of label over the inner spiral bundle tunnel spiral bundle and tunnel crossing fibers (Fig 1) In the second turn most outer hair cells in row 1 and some hair cells in rows 2 and 3 have a cluster of silver grains at their base (Fig 2) No clusters of silver grains are present at the base of outer hair cells in the apical turns Neither outer nor inner hair cells have a significant accumulation of grains over th Outer spiral bundles generally have no silver grains over them than surrounding porting cells Schwann cells in the bony s lip and habenula are always heavily lab Scattered silver grains are present over cellular structures in the organ of Corti surrounding regions including the stria culans and Reissner s membrane

Glutamate

As after incubation in ^{3}H-GABA the distr tion of silver grains over the organ of C after incubation in ^{3}H glutamate is heavy the inner spiral bundle and tunnel spiral bu (Fig 3) Label over the tunnel crossing fi and at the base of the outer hair cells is pre (Fig 3) but fewer hair cells and tunnel cros fibers are labeled than after incubation in GABA Label is not present over the habe or over the nerve fibers in the bony spiral The stria vascularis basilar membrane Reissner s membrane are generally hea labeled No significant accumulation of la is found over inner or outer hair cells

Aspartate

After incubation in ^{3}H aspartate the in spiral bundle and tunnel spiral bundle heavily labeled (Fig 4) No label is seen o either the tunnel crossing fibers or over base of outer hair cells As with ^{3}H glutama the stria vascularis and basilar membrane heavily labeled and inner and outer hair c have no significant label over them The he of the pillar cells have many silver grains o them (Fig 4)

Glycine

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Non-neural structures				
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Reissner' membrane	–	++	+	++
Habenula	+	–	–	–
Schwann cells	++	–		

The amount of label over each cochlear structure is expressed relative to background for that amino acid.

hair cells (Fig 5) These grains are evenly distributed over the entire cell including over the region of the cell which contributes to the reticular lamina. The efferent and afferent fiber bundles are unlabeled as are the outer hair cells

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Glutamate

As after incubation in ^{3}H-GABA the distribution of silver grains over the organ of Corti after incubation in ^{3}H-glutamate is heavy over the inner spiral bundle and tunnel spiral bundle (Fig 3) Label over the tunnel crossing fibers and at the base of the outer hair cells is present (Fig 3) but fewer hair cells and tunnel crossing fibers are labeled than after incubation in ^{3}H-GABA Label is not present over the habenula or over the nerve fibers in the bony spiral lip The stria vascularis basilar membrane and Reissner s membrane are generally heavily labeled No significant accumulation of label is found over inner or outer hair cells

Aspartate

After incubation in ^{3}H-aspartate the inner spiral bundle and tunnel spiral bundle are heavily labeled (Fig 4) No label is seen over either the tunnel crossing fibers or over the base of outer hair cells As with ^{3}H-glutamate the stria vascularis and basilar membrane are heavily labeled and inner and outer hair cells have no significant label over them The heads of the pillar cells have many silver grains over them (Fig 4)

Glycine

In general cells of the organ of Corti accumulate less label after incubation in ^{3}H glycine than after incubation in the other amino acids The stria vascularis and Reissner s membrane however are heavily labeled The heaviest accumulation of silver grains over the organ of Corti is over inner

any fibers synapse on the radial afferents which terminate on inner hair cells. In turns 2 and 3 efferent fibers cross the tunnel as tunnel crossing fibers and synapse on most outer hair cells in row 1 and some outer hair cells in rows 2 and 3 (Wright & Preston 1973). The number of tunnel crossing fibers decreases progressively towards the apex. The uptake of ^{3}H-GABA is heaviest in those regions of the organ of Corti containing efferent fibers and terminals. Like the distribution of efferent terminals on the outer hair cells, there is less labeling over the base of outer hair cells in the apical turns and row 1 outer hair cells are more consistently labeled than hair cells in rows 2 and 3. This pattern of labeling is similar to that described by Riehrath et al. (1974) after in vivo incubation of the organ of Corti in ^{3}H-GABA. The uptake of ^{3}H-glutamate is similar to that of ^{3}H-GABA although fewer outer hair cells in turns 2 and 3 have label over their base. Using ^{3}H aspartate, the inner spiral bundle and tunnel spiral bundle are labeled; however, no label was seen over the bases of any outer hair cells or tunnel crossing fibers.

The fact that GABA, glutamate and aspartate are taken up into efferents, which are almost certainly cholinergic, suggests that high affinity uptake of these substances is not restricted to terminals in which they are stored for release as neurotransmitter substances. It appears unlikely that high affinity uptake can be useful for identifying the neurotransmitter of the hair cell to afferent synapses.

Fig. 1. Autoradiograph of the 2nd turn of an organ of Corti incubated in ^{3}H-*GABA*. Label is present over the inner spiral bundle (*arrow*), tunnel spiral bundle (*arrow head*), tunnel crossing fibers (*small arrows*). Schwann cells in the bony spiral lip and habenula (*H*) are also heavily labeled. ×1000.

Fig. 2. Autoradiograph of the 2nd turn of an organ of Corti incubated in ^{3}H-GABA. A cluster of silver grains (*arrow*) is present beneath the base of an outer hair cell in row 1. ×2500.

Fig. 3. Autoradiograph of the 3rd turn of an organ of Corti incubated in ^{3}H-glutamate. In this autoradiograph, label is present over all cochlear structures, but is heavy over the inner spiral bundle (*I*), tunnel spiral bundle (*arrow head*), tunnel crossing fibers (*small arrows*) and beneath the base of a row 1 outer hair cell (*arrow*). ×800.

Fig. 4. Autoradiograph of the 2nd turn of an organ of Corti incubated in ^{3}H-aspartate. Heavy labeling is present over the inner spiral bundle (*arrow*) and tunnel spiral bundle (*arrow head*). The heads of the pillar cells (*star*) in the reticular lamina are also heavily labeled. ×800.

Fig. 5. Autoradiograph of the inner hair cell region of the 3rd turn of an organ of Corti incubated in ^{3}H-glycine. The inner hair cell (*I*) has more label over it than surrounding structures. The label over the inner hair cell is uniformly distributed over the entire cell. ×900.

Fig. 6. Autoradiograph of the 2nd turn of an organ of Corti incubated in ^{3}H-leucine. Label is uniformly distributed over all cochlear structures. ×900.

ACKNOWLEDGEMENT

We thank Mrs G. R. Nelses for her technical assistance in this project.

ZUSAMMENFASSUNG

Die Verteilung von Silberkörnern über dem Cortischen Organ des Meerschweinchens wurde in Autoradiogrammen nach Inkubation in vitro mit ^{3}H-markierten mutmaßlichen synaptischen Transmittoren lichtmikroskopisch untersucht. Nach Inkubation mit ^{3}H-Glycin fand sich eine kräftige Silberkornbelegung über den inneren Haarzellen, die aber nicht auf die synaptische Region beschränkt war. Inkubation mit ^{3}H-GABA, -Glutamat und Aspartat führte zu einer kräftigen Markierung der Fasern und Endigungen des efferenten olivo-kochleären Bündels. Leucin, das vermutlich kein Transmittor ist, wurde von allen kochleären Strukturen gleichmäßig aufgenommen. Die Tatsache, daß GABA, Glutamat und Aspartat von efferenten, höchstwahrscheinlich cholinergen Fasern aufgenommen werden, spricht dafür, daß „high affinity uptake" dieser Substanzen nicht auf die Nervenendigungen beschränkt ist, in denen sie als Transmittoren freigesetzt werden.

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STRUCTURAL CHANGES IN THE ORGAN OF CORTI OF THE GUINEA PIG AFTER OBSTRUCTION OF THE ARTERIAL BLOOD FLOW TO THE INNER EAR

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Abstract. Forty-one young guinea pigs were studied after obstruction of the anterior inferior cerebellar artery in order to ascertain the effects on the surface specimen of the organ of Corti. Most of the animals had damage to the third and fourth turns (22) and a minority of these had deafness and destruction nystagmus (3). Our hypothesis is that one of the causes of Menière's disease is an interference with the inner ear circulation, and our plans for testing this hypothesis are given.

A comprehensive study of the circulation in the inner ear of normal guinea pigs was made by Axelsson in 1968.

Several authors have studied the pathological changes and lesions in the receptor organs of the inner ear after experimental obstruction of the supplying and draining vessels. Kimura & Perlman (1956) found after acute venous obstruction in the guinea pig a degeneration of the sensory epithelium of the organ of Corti with a loss of outer hair cells which were more vulnerable than the inner hair cells. After acute arterial obstruction the damage was more pronounced than after the venous obstruction (Kimura & Perlman, 1958). Perlman et al. (1959) completed this study with intermittent closure of the internal auditory artery and they found histological changes in the labyrinth after one hour of obstruction. In none of these studies was any vestibular hydrops reported.

Degenerative changes in the organ of Corti occur most often in the basal turn, e.g. after treatment with ototoxic drugs (Hawkins & Lurie, 1953; Lurie, 1955; Benitez et al., 1962) and atrophic changes associated with ageing (Schuknecht, 1955; Schuknecht & Igarashi, 1964). On the other hand Alford et al. (1965) and Bernstein & Silverstein (1966) have shown changes in the apical turn of the cochlea after obstruction of the arterial supply. Bernstein & Silverstein (1966) have also revised the preparations from Kimura & Perlman (1958) and found the same damage in the apical turn. Bernstein & Schuknecht (1967) also showed lesions of the apical region of the cochlea after experimental traumata to the round and oval windows.

All these studies were made on conventional histological preparations. To make a more detailed description of the histological status of the neuroepithelial surface the preparation technique *ad modum* Engström et al. (1966) is more suitable.

The aim of the present study was to investigate the location and extent of damage in the organ of Corti after obstruction of the anterior inferior cerebellar artery in the guinea pig using the technique according to Engström et al. (1966).

MATERIAL AND METHOD

Forty-one healthy young guinea pigs, weighing about 250 grams, were used. Before the experiment their hearing was tested by Preyer's reflex and all the animals were found to have a normal reflex threshold.

The animals were anaesthetized by intra-

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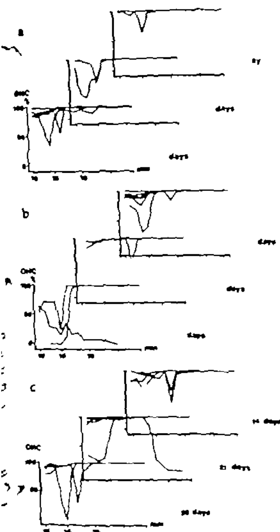


Fig. 2. A summary of the surface specimen from those 34 animals sacrificed 18 hours or more after the occlusion of the anterior inferior cerebellar artery covers 1–4 days, *b* 5–8 days and 14–28 days. OHC = outer hair cell. The hair cell loss is here graphically shown as percentage loss in each millimetre in relation to the location in the organ of Corti from base to apex.

rected towards the healthy ear. The majority of the animals were unsteady during the first postoperative hours but no nystagmus was seen.

DISCUSSION

Previous studies on the effects of obstruction of the anterior inferior cerebellar artery have shown damage in the apical turn of the cochlea (Alford et al. 1965, Bernstein & Silverstein 1966). These studies, however, were done on sectioned specimens which give no exact information on the location of and variation in the damage. By using a method with surface preparation a more detailed examination can be made.

In the present study the damage was found in the third and apical turn of the cochlea at a maximum of 15–17 mm from the base and leaving in most of the cases (17) the last apical mm intact. In the two cases with damage in the basal turn we strongly suspect that not only was the arterial supply blocked but the venous drainage as well. It seems that the damage can first be seen after 1? hours and the destruction increases during the first week after obstruction of the arterial supply. Even after an observation time of 4 weeks there were no pathological changes in the basal turn with the above-mentioned exceptions.

In 3 animals we found a pronounced loss of balance and a destruction nystagmus postoperatively and thus there must also have been an interference with the blood supply to the vestibular part of the inner ear.

This study has thus shown that obstruction of the anteriour inferior cerebellar artery gives rise in a majority of the animals to damage in the third and fourth turns of the organ of Corti. This part corresponds to a low tone loss *in the* audiogram.

There were 8 animals sacrificed *18* hours and later postoperatively which showed no damage to the organ of Corti. According to Axelsson (1968) there are some cases were a good collateral circulation is established and those animals with an intact organ of Corti after the obstruction of the arterial supply probably had such a circulation.

The results of this study on damage *to the* outer hair cells in the third and fourth turns of the cochlea in a majority of the animals and loss of balance with destruction nystagmus in some animals have raised the question whether *disturbances in* the circulation in the an-

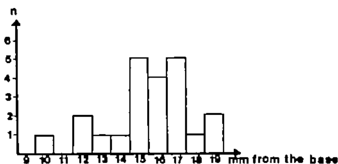


Fig 1 The location of the maximal destruction on the surface specimen in those 22 animals with a defined maximum

peritoneal injection of Nembutal 30 mg/kg body weight A submental incision was made The styloid process was exposed and fractured medially to give a good exposure of the auditory bulla After having removed the anterior wall of the bulla an excellent approach to the middle ear cavity was established By removing the medial wall of the bulla at the level of the basal turn of the cochlea the anterior inferior cerebellar artery was visualized under the dura. For good access to the vessel the dura was opened wide To ensure complete obstruction of the blood flow the vessel was avulsed Haemostasis was achieved with a piece of Surgicel The wound was closed with skin sutures

The animals were sacrificed after 3 6 12 18 and 24 hours and 3 4 5 7 8 14 21 and 28 days respectively after the operation After decapitation the temporal bones were removed and the tympanic bulla opened With the temporal bone submerged in the fixation fluid of 1 5% buffered osmium tetroxide the round window membrane was opened the stapes was extracted or pushed into the vestibule and through a small opening in the apex the cochlea was perfused with the fixation fluid The specimen was kept submerged in the osmium tetroxide solution for 2 hours and subsequently stored in 70% alcohol until micropreparation The preparation was performed under a stereo microscope and a surface specimen of the whole organ of Corti was made This technique is described in detail by Engström et al (1966)

The surface preparations were studied in a phase-contrast microscope and the whole organ of Corti from base to apex was investigated The hair cell loss was thoroughly registered for each row separately and is graphically shown as hair cell loss in each millimetre in relation to the location in the organ of Corti from base to apex

The non-operated ear served as a control for hereditary and acquired inner ear disease.

RESULTS

Among those 7 animals sacrificed within 12 hours after the occlusion there was no hair cell damage

Of the 34 animals sacrificed 18 hours or later after the occlusion 23 (67 6%) had more or less pronounced damage of the hair cells. Another 3 (8 8%) could not be studied because of damage during micropreparation at the usual location of the pathological findings The remaining 8 were intact and no damage of the hair cells was seen

Fig 1 shows the location of maximum damage of the hair cells in 22 of the 23 animals damaged The 23rd was excluded because the antire organ of Corti was destroyed Three of the 23 animals had additional changes one was totally destroyed from the 15th mm to the apex one also had a maximum at 11 mm and the third was also damaged from 1 to 8 mm. All the surface specimens are illustrated separately in Fig 2 *a–c* which also illustrates how the damage process continues during the first week after the obstruction

It seems that with exception of the 2 abovementioned animals no damage will occur from the 1st to the 9th mm whatever the time elapsed after the arterial occlusion

In all the damaged surface specimens the pathological changes were found in the oute hair cells while the inner hair cells seem almost resistant to the loss of blood supply from the anterior inferior cerebellar artery Three of the animals had a pronounced loss of balance postoperatively and a clear

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FURTHER STUDIES OF THE MEMBRANE POTENTIAL OF THE STRIA CELLS OF THE GUINEA PIG *IN VITRO*

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ract Negative membrane potentials from the stria ılaris of the guinea pig have been recorded *in vivo*. ıe negative membrane potentials from the stria cells also been recorded for as long as 2 minutes *in* They varied from −20 mV to −49 mV irrespective hether the stria vascularis was immersed in Ringer' ion or in K-Ringer solution (approximating to the position of an endolymph). Labelling of the inserted cells from which the membrane potential had been rded demonstrated that the microelectrode tip was e the cell. These cells will continue to show electrical ity for some time when immersed in suitable media.

: membrane potential of stria cells of the ıea pig *in vitro* has been found (Chou et al. 5) to be approximately −30 mV in both ger's solution and K Ringer's solution in ch sodium ions have been replaced by mM potassium ions The composition of latter medium may be regarded as approximating to that of an endolymph The ertion of a microelectrode into a stria cell always very difficult and since the stria s are small a high degree of accuracy is ential In the present series of experiments h *in vivo* and *in vitro* measurements have n made Electrodes with tip diameters not er than 0.5 μm have been used with the tion of the electrode tip located by electrophoretic dye injection To facilitate interpretation of the results the surface structure he stria vascularis has been examined using canning electron microscope

METHODS

o series of experiments were performed ng more than 50 guinea pigs (weighing 260–800 g) In order to measure the membrane potentials of the stria cells *in vivo* and *in vitro* For the latter two separate media were used. (1) Krebs-Ringer's solution with substrates, and (2) Ringer's solution in which Na is replaced by 150 mM K This medium was prepared according to the formula given by Kuijpers (1969) Substrates were added and the pH adjusted to 7 4 (Misrahy et al. 1958)

Electrodes

Electrodes were prepared by drawing out Corning glass, with an outer diameter of 1.5 mm (Supplier Ernst Keller & Co A G Glasapparat Fabric 4002-Basel Switzerland) using a vertical electrode puller (Modell GA 10286 Firma Heinz Albrecht, München FGR) Before drawing out the glass tube a fine glass fibre of about 25–40 μm in diameter was placed inside it This facilitated the subsequent filling with potassium chloride solution. As described by K. Tasaki and his co-workers (Tasaki et al 1968) the remarkable characteristic of electrodes filled by this method is their low electrical resistance The resistance of the electrodes was between 20 and 50 MΩ

The sizes of the electrode tips were measured with a ×100 flat-field objective but without using immersion oil, which would have contaminated them. The working distance of a ×100 objective is minute in the range 110–160 μm so that care must be taken to ensure that the lens does not touch the tip of the electrode Alternatively a ×40 objective with

terior inferior cerebellar artery might be part of the etiology of Meniere s disease Only 3 animals lost their balance and developed nystagmus though the majority of animals were somewhat unsteady postoperatively

Patients who do not have a good collateral circulation in the apical part of the cochlea might possibly develop Meniere s disease if the blood flow in the anterior inferior cerebellar artery is interupted A spasm in the supplying vessel could then lead to an attack of vertigo combined with a low tone loss

In order to test the hypothesis that intermittent obstruction of the arterial blood flow is a possible etiological factor in Ménière s disease we are trying to find a method by which to achieve intermittent obstruction of the vessel in conscious animals Further we intend to perform bilateral obstruction and test the hearing on the assumption that these animals have a loss of their basal hearing capacity The study will be completed with nystagmographic examinations during and after the arterial obstruction

ZUSAMMENFASSUNG

41 Meerschweinchen wurden auf oberflächliche Veränderungen im Cortischen Organ untersucht nach Abklemmung der Arteria cerebellaris anterior inferior Die meisten Tiere zeigten Läsionen in der dritten und vierten Windelung; darunter befanden sich welche die auch Schwindel und Destruktionsnystagmus zeigten Unsere Hypothese ist demnach daß eine Erklärung für die Menièresche Krankheit in einer Kreislaufstörung im Innerohr liegen könnte Unsere Pläne für die Untersuchung dieser Hypothese werden erläutert

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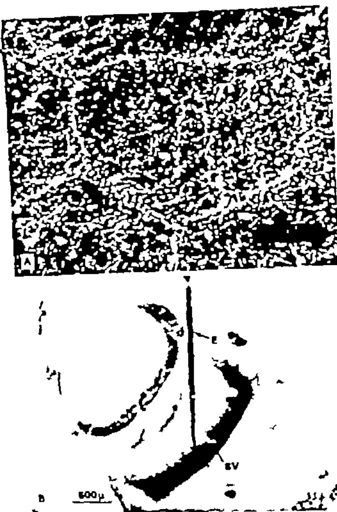


Fig. 2. (A) Scanning electron microscope photograph of the surface of the stria vascularis of guinea pig, to show the surface prominences (*P*) and numerous microvilli. (B) A cochlea which has been cut in half with the pigmented stria facing upwards. A recording electrode (*E*) is held in vertical position, with its movement during insertion in the direction of its axis and perpendicular to the surface of the stria cell (*SV*).

(*q*) 30 mm in diameter and 10 mm deep was used to hold the opened cochlea which had to remain fully immersed. The external cochlear wall (*p*) was firmly fixed to the base of the trough by means of a small drop of *histoacryl blue* (Supplier: B. Braun Melsungen A.G. 3508 Melsungen FGR). Care was taken to ensure that the stria vascularis was facing upwards in the manner shown in Fig. 2B. The trough attached to the Jolter (*j*)—details of this instrument are given in Chou et al. 1975—and an illuminated glass fibre (Ø 5 mm) (*l*) was placed just below the trough and specimen. This method of illumination avoids any heating of the specimens by the light source or reflection of light by the medium. The stria vascularis, attached to the inner wall of the cochlea, was observed through a binocular microscope.

The trough contained about 3 ml of the medium (*m*) which was changed continuously except during recording. The recording was carried out at 36°C, a temperature close to the guinea pig's body temperature, with a few readings taken at room temperature. The whole procedure from removal of the half cochlea to the first recording from a stria cell can be easily accomplished within 5 minutes.

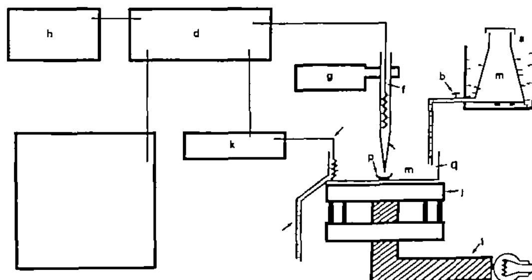


Fig 1 Diagram of apparatus used for electrical recording from the stria vascularis of the guinea pig *a* medium reservoir in thermostat, *b* screw clip on inflow lead, *c* outflow *d* amplifier and bridge *e* microelectrode *f* silver–silver chloride wire *g* Leitz micromanipulator *h* stimulator; *j* Jolter *k* voltage calibrator *l* illuminated glass fibre *m* medium *n* reference electrode *o* oscilloscope and electronic recorder *p* specimen: cochlear wall and stria vascularis, *q* trough

a working distance in the range 200–600 μm can be used In this case the tube length of the microscope must be extended in order to obtain a clear reading with the least diminution of quality of the image The tips of the electrodes were measured and examined immediately before use so that any with a very fine tip that had become blocked by small air bubbles could be discarded According to this method the measured external diameter of the electrode tips used in these experiments were about 0 6–0 8 μm but because of the quality of the image the apparent size as measured is subject to considerable error

It is desirable to have a reliable measurement of the exact size of the electrode tip Representative samples of electrodes were therefore submitted to Dr L Schmidts and Dr M Schneider of Senckenbergisches Zentrum der Pathologie of the J W Goethe Universität Frankfurt a Main for examination in the scanning electron microscope With their permission two photographs of typical electrodes are reproduced in Fig 4 (C D) They show that the electrode external diameters at the tips are about 0 28 and 0 38 μm

In vivo

The technique for exposing the cochlea and inserting the electrode through the spiral ligament and stria vascularis into the scala media has been described in a previous paper (Chou et al 1975) The microelectrode was mounted on the holder of a Leitz manipulator The fine vertical mechanical drive of the manipulator performed a very exact movement One small division was calculated to be equivalent to 1 μm The rate of electrode insertion (from the small hole made on the cochlear wall through spiral ligament and stria vascularis) was co-ordinated with the speed of the electronic recorder For example a 25 μm advance of the electrode tip corresponded to a recording distance of 1 cm in 15 seconds

In vitro

The apparatus and electrical circuit for *in vitro* experiments are shown diagrammatically in Fig 1 The cochlea was exposed and cut in half so that the part with intact stria vascularis but without modiolus could be immersed immediately and thoroughly washed in one or other of the two media A trough

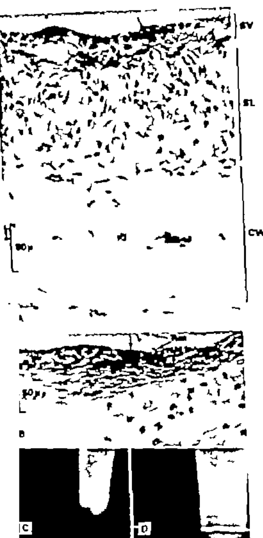


Fig. 4. (A) Stria vascularis of the guinea pig, from the basal turn of the cochlea, labelled with potassium ferrocyanide. The dark spot (↑) on the right was produced by electrophoretic dye-injection, to identify a stria cell. The tissue was cut longitudinally (10 μm). (B) Stria cell near the Reissner' membrane was intensely marked by electrophoretic dye-injection (↑). *RM*: remnant of Reissner' membrane. *SV*: Stria vascularis; *SL*: spiral ligament; *CW*: cochlear wall. Transverse section from the second turn of the cochlea (10 μm). (C, D) Photomicrographs of typical electrodes obtained with scanning electron microscope. The gap in the horizontal line represents 0.5 μm. *C*: tip diameter = 0.28 μm. *D*: tip diameter = 0.38 μm.

(3) Slow: about 25 μm per second or less. A progressive negative deflection was always observed before the rise to the positive endocochlear potential (Fig. 3 A).

In 86 measured recordings from 86 different cochleae, when glass microelectrodes filled with 3 M KCl were inserted slowly there was always a negative deflection before the positive endocochlear potential was recorded. The average value of this negative potential was −12 mV with a maximum value of about −17 mV.

Fig. 3 A shows a recording of the endocochlear potential from the basal turn of the cochlea. The electrode passed through an aperture made in the cochlear wall, the spiral ligament and stria vascularis. A progressive negative potential was observed just before the positive potential. The negative potential (that in Fig. 3 A is about −10 mV) indicates that the electrode tip was within tissue. (N.B. In these recordings the time runs from the right to the left.)

II. *Electrodes filled with Isotonic KCl (110 mM)*

In these experiments from 30 cochleae the expected negative potential when the electrode was passing through the stria vascularis was not observed. Positive endocochlear potentials were of the order of 70 mV (Fig. 3 B).

Membrane potential of Stria Cells in vitro

Although *in vivo* there was a most convincing relationship between the observed structure and the negative potential inside the stria vascularis the exact correlation remained in doubt. *In vitro* experiments were therefore carried out with the apparatus already described. Before presenting these results, one feature of the material and a technique must be briefly described.

Surface structure of the stria vascularis

When viewed in the scanning electron microscope it can be seen that the surface of the stria vascularis is not smooth (Fig. 2 A). There are prominences on the surface approximately 1.5 μm in diameter as well as numerous microvilli

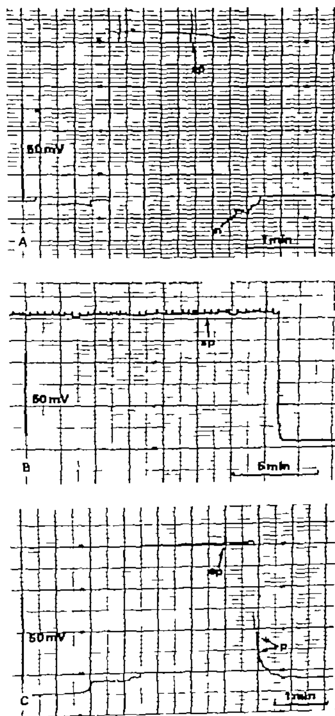


Fig 3 Typical recordings of the endocochlear potential from the scala media of guinea pigs The small spikes (*sp*) shown in all three recordings correspond to the movement of the tympanic membrane (N.B The time runs from right to left on the recordings) (A) Electrode filled with 3 M KCl There is always a negative potential (*n*) just before the registration of the endocochlear potential. Rate of insertion 25 μm/15 sec Electrode tip diameter approx 0.4 μm. (B) Electrode filled with 110 mM KCl No negative potential was observed before the positive endocochlear potential. Rate of insertion 25 μm/15 sec. Electrode tip diameter approx 0.4 μm (C) Electrode filled with 3 M KCl. The tip diameter was approx 5 μm. Distinct positive steps (*p*) are shown until the endocochlear potential registered Rate of insertion 25 μm/15 sec

Recording Equipment

This is illustrated in Fig 1 The glass micro electrode—with a silver–silver chloride wire (*f*)—was connected to a Keithley 604 differential amplifier or a bridge circuit (*d*) and the reference electrode (*n*) was placed in the medium The output was displayed on a Tektronix oscilloscope 5113 or registered on an electronic recorder (*o*) Usually a recording speed of 4 cm per minute was used. The voltages of the oscilloscope and electronic recorder were accurately calibrated (*k*) The stimulator (*h*) was used to provide pulses for measurement and checking of the electrode resistance before and after insertion

RESULTS

Measurements of Endocochlear Potentials and Membrane potentials of Stria Cells in vivo

Throughout the investigations it was observed that the results obtained were dependent upon (1) the diameter of the electrode tip (2) the concentration of the electrolyte filling the electrode and (3) the rate at which the electrode was inserted into the scala media through the spiral ligament and stria vascularis.

Two electrolyte concentrations were used for the study of the positive endocochlear potential and membrane potential of stria vascularis cells *in vivo* 3 M KCl and isotonic (110 mM) KCl solutions

I *Electrodes filled with 3 M KCl*

These were inserted through the spiral ligament and stria vascularis into the scala media at different rates

(1) Fast in the order of 150 μm or more per second No deflection or very small negative deflection was observed before the positive endocochlear potential
(2) Moderately fast 50 μm per 15 sec to 75 μm per 15 sec A negative deflection was always observed before the positive endocochlear potential

higher than 150 mM Microanalysis by flame photometry has been used to determine the contents of dried stria tissue but the technique for obtaining uncontaminated stria still needs to be improved

Membrane potentials that are insensitive to the external potassium concentration are known to exist. For example the membrane potentials of somatic muscle cells from *Ascaris* are insensitive to the external potassium concentration (del Castillo et al 1964 Brading & Caldwell, 1964 1971) So is the *Nitella* (Kitasato 1968) Muscle fibres can make their membrane potentials insensitive to external concentrations of potassium by equilibration in Ringer s solution that contains higher concentrations of potassium (Beague et al. 1975)

In the present series of experiments an unstable positive potential was frequently observed before the microelectrode was properly inserted into a stria cell This was followed by a stable negative potential or sometimes a small unstable negative potential as shown in Fig 5 E. The unstable positive potential is believed to be an insertion artefact caused by bending of the electrode tip or otherwise subjecting it to stress prior to insertion through the cell membrane Since the reading was made *i tro* such a positive potential (or positive steps) could not be related to the positive endocochlear potential.

A small unstable negative potential (see Fig 5 E c) was observed fairly often. This appeared to be because the electrode was inserted in the position of one of the prominences shown in Fig. A. This interpretation was supported by the height of the negative potential as well as its instability Since the Jolter instrument gives only a small forward vibration of 1– μm each time the electrode tip (with a diameter of less than 0.5 μm) could not have been brought into contact with any other structure When the microelectrode was properly inserted into a stria cell there wa always an abrupt deflection followed by a table negative membrane potential (Fig. 5 E, d) When the electrode was finally withdrawn from the cell (*e*) the recorded potential returned to zero

Comparison with other findings

Prazma (1975) reported that during penetration of spiral ligament he recorded a small negative potential in the range of 4–20 mV As the electrode approached the basal cells of the stria vascularis the potential reversed its polarity to positive readings He concluded that the marginal cells of the stria facing the endolymphatic space have a positive intracellular potential similar to the potential of the scala media. Unfortunately in his photographs a standard scale was not provided and the particular turn of the cochlea from which the recordings were made was not mentioned With the recording method he has described the depth of penetration of the electrode should correspond approximately to the thickness of the stria vascularis but the positive potential area in his recordings varied widely from 10 μm to 3? μm Although the thickness of the stria vascularis is not uniform in the optimal position for insertion (which is in the central region of stria–spiral ligament) in the first or second turn of the cochlea (were a small hole can be made through which the electrode can be advanced into the endolymphatic space) the thickness is in the range 25–37 μm This is for guinea pigs 8 months to 2 years old of body weight 230–600 grams. It would thus seem that the cause of those unstable positive potentials encountered before the electrode had definitely penetrated into the endolymphatic space must have been factors other than the so-called positive intracellular potential of the stria cells

Similar vague positive steps were reported by Sellick & Johnstone (1975) To quote Electrode penetrations of the stria from the serosal side *never show a negative potential only vague positive steps* until EP is recorded. Attempts to penetrate the cells from mucosal side have been largely unsuccessful however *some negative potentials of about −30 mV were recorded* but were not stable enough to

DISCUSSION

The membrane potential of the stria vascularis was always negative irrespective of whether the measurement was carried out *in vivo* or *in vitro*. The method of approaching the stria cells *in vivo* described is far from satisfactory since the microelectrode must pass through the bony aperture and spiral ligament so that each time as the fine tip is inserted through a cell it is liable to be damaged. However the method does have the advantage that anatomically the stria vascularis is the only tissue in the immediate vicinity of the scala media and the positive endocochlear potential can be taken as an indication of where the electrode is located. The chart records (Fig 3 A B C) show clearly the positive potential that is universally accepted as that of the endocochlear potential. The spiral ligament which is sandwiched between the cochlear wall on the outside and the stria vascularis on the side of the scala media, consists of connective tissue cells and bundles of fibrils that are loosely held together (Spoendlin 1970) with vessels also present (Smith 1957) and no potential was recorded in the spiral ligament. The observed negative potential indicates therefore that the electrode tip was truly in the stria vascularis. This observation confirms the report by Békésy (1952*a* 1952*b* 1960) that "inside the stria cells [the potential] was *always negative* but the ligamentum spiralis did not show potential difference against the perilymph.

Distinct positive steps (or recordings) were often observed when an electrode with tip diameter larger than 3 μm was used for recording the endocochlear potential (Fig 3 C). The reason is apparent since an electrode with such a large tip can be expected to cause general damage to the cell membrane during insertion while the pressure on the stria tissue as a whole is far greater than that produced by a microelectrode with a fine tip acting on a small area of the cell membrane (Békésy 1952*a*).

When the medium for the *in vitro* method is carefully prepared with the medium saturated with oxygen and essential substrates present the stria can maintain its activity. It was shown by tissue respiration studies some time ago (Chou & Rodgers 1962) that when the oxygen supply for the isolated stria vascularis is adequate it remains in an active state. The stria and many other tissues (e g. cortex of kidney liver coroid plexus etc) can maintain their active oxygen consumption in Ringer's solution in a Cartesian diver for at least 40 minutes. Three *microlitres* was sufficient medium to maintain one small piece of tissue for the Cartesian diver method. It had not previously been demonstrated that the stria vascularis showed a high rate of oxygen consumption *in vitro* although it is usually referred to as active.

The negative potential recorded from the stria vascularis revealed two significant facts:

(1) that with the technique that has been used the electrode is placed truly within a stria cell. The recorded potential could n possibly be due to an artefact (see Fig 4 B)

(2) the potential of the stria cell is negativ with respect to the extracellular medium

The negative membrane potential observe when K Ringer's solution was used as th medium suggests but does not prove that th concentration of potassium ions within th cells might be higher than that of the sur rounding medium. It is well known that pota sium can depolarize the membrane potenti of muscle fibres. The linear relation betwee the membrane potential and the logarithm (the external potassium concentration has bee taken as evidence that this potential aris from the difference between the concentr tions of potassium ions inside and outside th cell (Höber 1905 Bernstein 1912 Hodgki 1951). This interpretation could also be a plied to stria tissue if the internal ionic co centration of potassium in the stria cells we

ellen zeigten wahrend der Experimente *in vitro* elektrische Aktivitat, wenn sie sich in einem Medium mit entsprechenden Bedingungen befanden

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observe their behaviour during anoxia (Johnston unpublished) (Sellick & Johnstone 1975 p 357 para 2) They did not say what concentration of electrolyte was in their electrodes their interpretations seem to lack clarity and as the print in italics shows are mutually contradictory To assess their results further we would need to have more complete details of the solutions they used their method of insertion of the electrodes *in vivo* and the original recordings The necessity for using electrodes filled with 3 M KCl to record intracellular potentials is well known and was explained in detail by Nastuk & Hodgkin (1950) Békésy (1952*a*) also strongly recommended use of this concentration for recording the endocochlear potential

A positive potential on the surface of the stria vascularis was first demonstrated by Tasaki & Spyropoulos in 1959 They moved the tip of their recording electrode along over the exposed surface of the stria A positive potential of the order of 10 mV was measured on a few occasions rising to 30–55 mV They observed that the sharp edge of the glass pipette electrode could damage the cells it came in contact with and so distort the potential At that time the prominences on the surface of the stria vascularis shown in Fig 2A were not known although the presence of microvilli had been demonstrated by electron microscopists Their electrode tip was about half the size of a stria cell so that by moving it along the surface of the stria tissue those prominences would inevitably have been touched or damaged Because of the recorded value of +10 mV with occasional values of +30 mV to +55 mV they came to the conclusion that the endocochlear potential (+80 mV) is produced by cells in the stria vascularis However it would be difficult to explain how stria cells possessing only +30 mV or +55 mV could produce and maintain a stable endocochlear potential with a value of +80 mV

In the *in vitro* experiments positive potentials of about 8–12 mV were often observed before the stable membrane potential from the stria cells was recorded (see Fig 5E) A similar positive potential is also observed when an electrode tip is forced through a muscle fibre Such positive potentials are also noticed if a thin layer of connective tissue covers the muscle or the movement of the electrode is not exactly parallel to the axis of the electrode during insertion Vague positive steps from such causes or the +30 mV to +50 mV potential from the surface of the stria cells are thus not directly related to the production of the +80 mV endocochlear potential In his thesis Kuijpers (1969) presented what seems to be satisfactory evidence for the suggestion that the stria vascularis is the most likely site for the production of the endocochlear potential

The main purpose of the work reported in this paper has been to demonstrate that there are stable negative membrane potentials in the stria vascularis Concentrations of potassium up to 150 mM did not affect the membrane potential *In vitro* the stria cells continue to show electrical activity for some time when immersed in suitable media

ACKNOWLEDGEMENTS

This work was supported by grants from Deutsche Forschungsgemeinschaft and Klinikum der J W Goethe Universitat Frankfurt am Main We are indebted to Mr B Jackson for his valuable suggestions to Miss Proctor for her secretarial help and to Miss Dirnberger for her technical assistance We would like to thank Dr H Waldvogel of Firma Carl Zeiss Frankfurt am Main for providing facilities to use the Novascan Raster Electronmicroscope (for Fig 2A) to Dr L Schmidts and Dr M Schneider for examining electrodes with the scanning electron microscope We are particularly grateful to Dr K Little of Oxford and Dr K F A Ross Reader in Anatomy Aberdeen University Scotland for their helpful advice and correction during the preparation of this manuscript

ZUSAMMENFASSUNG

E ist gelungen negative Potentiale der Stria vascularis an einem lebenden Meerschweinchen abzuleiten. *In vivo* dagegen schwankten d e Ergebnisse zwischen −70 mV to −49 mV sowohl Ringerlosung al uch n künstlicher Endolymphe Anschließend M rkierung der Stelle bewies daß sich die Spitze d r Elektrode während der elektrischen Ableitungen [illegible]

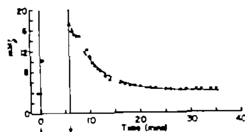


Fig 1 The response of potassium-ion electrode in the second turn of scala tympani (*ST*) to 6 min 20 mM K perfusion at 14 µl/min through *ST* The curve fitted to the data is only an approximation to the more complex recovery repeatedly observed. Under perfusion conditions the K electrode readings are always lower than the concentration of the solution being perfused This is difficult to explain on the basis of electrode behaviour entirely

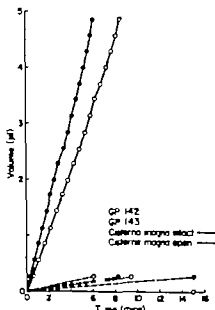


Fig 2 Cumulative displacement of perilymph from the basal turn of scala tympani measured using hydrophobic pipettes Dashed lines indicate the effect on fluid production rate by surgically releasing cerebrospinal fluid pressure in 2 animals (open closed circles). At low volume production rates consecutive measurements from the same preparation are plotted

tion of that used by Moscovitch et al. (1973) Long pipettes were pulled from uniform capillary tubing and their tips broken to 50 µm O D A calibration scale was attached to the pipette body and the inner surface was made hydrophobic with 2% Repelcote (Hopkin & Williams) A short side arm which was attached to the pipette 4 cm from the tip was connected to a micrometer-driven syringe and the assembly was filled with Ringer s solution. The syringe was used to locate the fluid meniscus in the calibrated arm at a suitable point and to return it there after a period of fluid efflux measurement. The tip of the micropipette was fitted tightly into the hole drilled in scala tympani and the animal was orientated such that the measuring arm of the pipette assembly was held horizontally

Fluid production rates were determined by timing the meniscus movement over millimetre segments Results were discarded if after the measurement period any sign of leakage was apparent around the point of insertion of the pipette into the cochlea. The rate measured under these conditions was compared with that measured in the same preparation immediately after the pressure exerted by the CSF had been released by surgically opening the cisterna magna This was performed by exposing the foramen magnum and rupturing the dura mater at this site by means of a dural hook. Care was taken to maintain the patency of the slit.

RESULTS

Ringer s solution containing elevated potassium levels were perfused usually at rates of 14 µm/min for a period of 6 min using a Braun perfusion pump These perfusion parameters were found to be optimal for obtaining a stable K concentration within a short enough time but without causing any mechanical effects on responses by the perfusion pressure Ion-sensitive electrodes sited in the second turn of ST measured K concentration and showed that a stable concentration was reached within 2 min At the end of the perfusion the pump was turned off and the replacement of the perfusate was allowed to occur spontaneously When monitoring the

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THE EFFECT OF CEREBROSPINAL FLUID PRESSURE ON PERILYMPHATIC FLOW IN THE OPENED COCHLEA

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(Received November 6, 1978)

Abstract Scala tympani of guinea pigs was perfused with elevated potassium solutions whose concentrations were measured with ion-sensitive pipettes It was noted that, upon spontaneous recovery the concentration curve showed a break approximately 3 min after perfusion ceased When the CSF pressure was released by opening the cisterna magna, cochlear flow was markedly reduced, and the recovery curve became smoothly exponential following a much slower return to control levels This finding lends support to the idea proposed by Moscovitch Gannon & Laszlo (1973) that longitudinal flow of CSF contributes to perilymph efflux in the patent cochlea.

In studies in which we artificially raised the potassium concentration in scala tympani (ST) (Salt & Stopp 1979) we observed that during spontaneous recovery the K concentration did not follow an exponential curve as one might have expected This observation has led us to study the mechanisms involved in regulating potassium concentration and has involved experiments which throw some light on the validity of two major hypotheses which are current namely that perilymph originates from cerebrospinal fluid (Altmann & Waltner 1947 1950 Gisselsson 1949) or alternatively that it arises as a blood ultrafiltrate in the perilymphatic capillary bed (Citron Exley & Hallpike 1956 Palva & Raunio 1967 Schnieder 1974)

The guinea pig is commonly used for cochlear studies including those of perfusion Its aqueduct has a fairly wide lumen and contains less connective tissue than is found in most other species therefore it is possible for bulk flow to occur from this reservoir into the perilymphatic space of scala tympani Moscovitch et al (1973) suggested that in cochlear perfusion experiments when the cochlea is patent such a longitudinal flow of CSF through the aqueduct could be a complicating factor We decided to test this idea by opening the cisterna magna to release the CSF pressure and comparing the efflux rates from the basal turn of ST before and after this manoeuvre This paper reports these findings.

METHODS

Guinea pigs weighing from 400 to 700 g were anaesthetized with 1 2 g/kg urethane injected intraperitoneally They were tracheotomized but allowed to breathe spontaneously and their normal body temperature was maintained throughout the experiment

The techniques involved for perfusing and recording from the cochlea are identical with those described in an earlier paper (Salt & Stopp 1979) except that in the efflux studies only a single hole was made in ST that being located approximately 2 mm from the round window

The method used to measure the rate of fluid efflux from the cochlea was a modifica-

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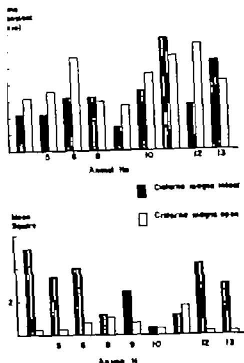


Fig. 4. Top. A comparison of the time constants for K concentration recovery before and after cisterna magna was opened in nine preparations. Recovery is from perfusion of 20 mM K solution through scala tympani at rate of 14 µl/min for 6 min. The mean of the time constant with the cisterna intact is 6.7 min and that after the cisterna was opened is 8.6 min. *Bottom.* The corresponding mean square deviations of the data points from the fitted curves for each of the above animals.

fitted curves to characterize the recovery process. The upper graph of Fig. 4 shows the change in recovery rate for individual animals. The mean value of the time constant for K concentration recovery in these nine experiments was 6.7 min with the cisterna intact, and 8.6 min with it opened. This difference is significant at $p<0.05$. It should also be noted that the mean square deviations of the data points from the fitted curves were significantly reduced (*F*-test, $p>0.01$) by the operation. This indicates that after the operation the potassium recovery curves were significantly closer to smooth exponential declines than before operation and supports the suggestion that the compound appearance of the curves may well be related to the presence of longitudinal flow of CSF along ST which Moscovitch et al. proposed.

DISCUSSION

Our experiments in which the rate of fluid efflux from the perforated ST was measured before and after release of CSF pressure strongly support the hypothesis put forward by others (e.g. Gisselsson 1949, Moscovitch et al. 1973) that this efflux takes place as a result of CSF entering the cochlea via the cochlear aqueduct. It is presumed that this flow arises as the direct consequence of the perforation of the otic capsule which releases the perilymphatic pressure and generates a pressure gradient across the cochlear aqueduct. We have shown that this flow significantly affects the time course of the recovery after the normal perilymph composition has been changed by perfusion. The potassium sensitive electrodes in our experiments were for practical reasons placed in the second turn. The break which we observed in the recovery curves would therefore be explicable in terms of the CSF having entered the cochlea at a single point (the cochlear aqueduct) and having taken a few minutes (the time to the break) to displace the contents of the ST between the aqueduct and the electrode. It was unfortunately not practicable to get a recording electrode close to the perfusion pipette to verify this hypothesis. *The calculated time to displace the contents of the lower turn at the rate of efflux measured would be nearer 6–8 min than the ~3 min observed. However as Moscovitch et al. discussed, it is possible that the volume production measured may be an underestimate of the actual efflux under these conditions and the possible ineffectiveness of perfusion in the fluid between the cochlear aqueduct and the perfusion pipette (the hook region) could make the effective space to be cleared even smaller.*

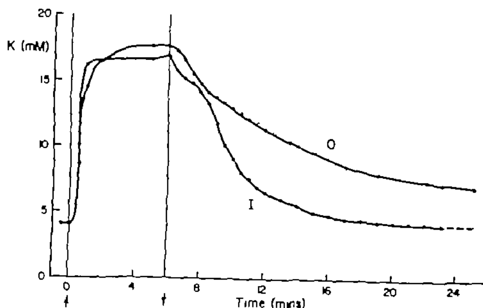


Fig 3 The effect of releasing the CSF pressure on the time course of recovery from a 70 mM K perfusion in one preparation O open cisterna magna, I intact preparation. Potassium concentration was measured with an ion sensitive electrode in ST II A 14 μl/min perfusion was for a duration of 6 min, as indicated by the arrows.

K^+ recovery curve we noted that it was not smooth but showed a break approximately 3 min after perfusion had ceased (Fig 1) As this pattern occurred consistently it suggested to us that two processes might be involved in the recovery and we therefore attempted to separate these components by releasing the CSF pressure by opening the cisterna magna.

The values for cochlear fluid efflux with cisterna magna intact were between 0 23 and 0 7 μl/min which are of the same order of magnitude as the 0 8–1 0 μl/min recorded by Moscovitch et al However these rates were dramatically reduced to between 0 and 0 045 μl/min (mean 0 016) after CSF pressure had been released (Fig 2)

It is possible that if the cochlear fluid originates as an ultrafiltrate of blood then any reduction in the blood pressure taking place as a result of our manipulation could have given rise to the effect observed However the mean arterial pressure as measured in the left carotid artery was found to be unchanged by the operation so that no general change in blood pressure could have produced the effect observed These findings therefore add support to the hypothesis that the efflux of fluid from ST when the cochlea is opened to atmospheric pressure indeed originates from CSF which has entered the scala due to the pressure gradient across the cochlear aqueduct

To determine what effect this introduction of CSF into the cochlea might have on the concentration of solutes in ST and especially on the recovery process we monitored potassium levels during and following perfusion in both the intact and opened cisterna magna conditions

Fig 3 illustrates the results of one such experiment While the perfusate was being introduced (at 14 μl/min) there was an insignificant difference between the concentrations recorded with the cisterna intact and subsequently with it open However as soon as perfusion ceased the curves under the two conditions began to deviate from each other almost at once The recovery curve in the intact preparation shows the characteristic two-slope feature referred to previously whereas that in the opened' condition not only shows a much slower return to control level but exhibits a smooth approximately exponential decline

To try to make a quantitative comparison between these two situations exponential curves were fitted to the recovery points using a least squares fitting routine We used the time constant of the recovery and the mean square deviation of the data points from the

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THE UPTAKE OF METHYL MERCURY IN GUINEA PIG COCHLEA IN RELATION TO ITS OTOTOXIC EFFECT

Teruzo Konishi and Philip E Hamrick

From the National Institute of Environmental Health Sciences Research Triangle Park, North Carolina USA

(Received September 8, 1978)

Abstract Guinea pigs were treated for 7 days by daily subcutaneous injection of methyl mercury chloride labeled with ^{14}C, the total dose of which was 17 mg Hg/kg. In these animals the cochlear microphonics and whole-nerve action potentials were suppressed in the basal turn but there was no marked losses in the third turn of the cochlea. The endocochlear potential was not decreased in magnitude. At the end of the treatment there was no accumulation of mercury in the perilymph, endolymph and cerebrospinal fluid. Uptake and elimination of mercury in the cochlear fluids were studied in guinea pigs which were treated by single intravenous injection of ^{203}Hg-labeled methyl mercury the dose of which ranged from 0.2 to 17 mg Hg/kg The results indicated that mercury concentration ratio of the blood relative to cochlear fluids was comparable with the blood to plasma ratio reported previously In contrast to lack of accumulation in the extra cellular environment, it is likely that tissues of the sensory end organs in the cochlea accumulated methyl mercury

The toxicity of methyl mercury was first described by Hunter et al. (1940) and Hunter & Russel (1954) Since several outbreaks of mass poisoning have recently been reported (Takeuchi 1972, Löfroth 1969 Kurland et al 1960), organic mercury compounds have come to be considered as dangerous pollutants. Otoneurological symptoms resulting from methyl mercury intoxication (known as Minamato disease) have been reported by Nosaka et al. (1970) and Fujisaki et al (1971) They include ataxia, loss of hearing and poor speech discrimination Morphological studies in guinea pigs indicate that the sensorineural hearing loss is caused by the degeneration of hair cells of the organ of Corti (Falk et al 1974). Although numerous reports have been published dealing with uptake and distribution of organic mercury compounds in various organs (Berlin & Ullberg, 1971 Suzuki et al. 1963 Yoshino et al 1966 Swensson & Ulfvarson, 1968 Takeda et al 1968 Norseth & Clarkson 1970 Piper et al. 1971 Kameda, 1972) none of these furnishes data on the distribution of methyl mercury in the cochlear fluids and there is little information available regarding the relationship between the magnitude of the cochlear potentials and the distribution of methyl mercury in the cochlear fluids

The experiments to be reported here were designed to investigate the distribution of methyl mercury labeled with ^{203}Hg and/or ^{14}C and relate its distribution to changes in the cochlear potentials in guinea pigs.

METHODS

Healthy guinea pigs (NIH strain) of about 250 g weight were used as experimental animals The control and treated animals were drawn from the same litter The experiments were carried out under anesthesia with pentobarbital sodium. The present investigation involves two separate experiments In the initial experiments guinea pigs were injected intravenously with a single dose of ^{203}Hg-labeled methyl mercury chloride (0.2 mg Hg/kg, 1 7 mg/Hg/kg and 17 mg Hg/kg) as a tracer to study the kinetics and distribution of mercury in the blood perilymph endolymph and cerebrospinal fluid (CSF). In the second experi

The potassium concentration commences to fall immediately on cessation of perfusion before the CSF plug could have reached the recording electrode and indeed recovery still takes place at a finite rate in the virtual absence of any CSF efflux (cisterna magna open) This strongly suggests that a local mechanism exists with normally determines the perilymphatic composition at least as far as potassium concentration is concerned Our measurements yield a time constant of 8 6 min for this mechanism which in terms of the cochlear half life proposed by Schnieder (1974) corresponds to a value of about 5 9 min for the half life of the potassium recovery process

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ZUSAMMENFASSUNG

Die Scala tympani bei Meerschweinchen wurde mit erhöhten Kaliumlösungen perfundiert, deren Konzentrationen mit ionenempfindlichen Pipetten gemessen wurden. Es wurde beobachtet daß die Konzentrationskurve nach spontaner *Erholung eine Unterbrechung ca. 3* Minuten nach Perfusionsende zeigte Nachdem der Druck des Liquors cerebrospinalis durch Offnen der Cisterna *magna* vermindert wurde ist der Fluß durch die Cochlea geringer geworden und die Erholungskurve glatt exponential geworden sie kehrte viel langsamer zu den Kontrollniveaus zurück Dieses Ergebnis spricht für die Theorie von Moscovitch Gannon & Lazlo (1973) daß in der normalen Cochlea der hinausgerichtete Fluß des Liquors cerebrospinalis zu dem perilymphatischen Abfluß beiträgt

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Healthy guinea pigs (NIH strain) of about 250 g weight were used as experimental animals The control and treated animals were drawn from the same litter The experiments were carried out under anesthesia with pentobarbital sodium. The present investigation involves two separate experiments In the initial experiments guinea pigs were injected intravenously with a single dose of ^{203}Hg-labeled methyl mercury chloride (0.2 mg Hg/kg, 1 7 mg/Hg/kg and 17 mg Hg/kg) as a tracer to study the kinetics and distribution of mercury in the blood perilymph endolymph and cerebrospinal fluid (CSF) In the second experi-

ment a total of 17 mg Hg/kg of [14]C labeled methyl mercury chloride was injected in tramuscularly in seven doses over a period 7 days While receiving volatile radioactive methyl mercury the animals were housed in side a chemical hood (Klein & Herman 1971) In the acute experiment [203]Hg-labeled methyl mercury chloride was injected into the super ficial jugular vein In experiments with re peated injection of [14]C labeled methyl mercury chloride the solution was injected intramuscu larly The [14]C labeled methyl mercury chloride was dissolved with 0 02 M Na_2Co_3 and the [203]Hg labeled methyl mercury chloride was already dissolved in 0 02 M Na_2Co_3 as purch ased The control guinea pigs were treated with intramuscular injection of 0 02 M Na_2Co_3 solution

A liquid scintillation counter was used to determine the concentration of [14]C and [203]Hg isotopes in both single and dual labeled mode Greater than 40% efficiency was maintained even for the most highly quenched samples and the efficiency was usually greater than 50% The activity in the endolymph peri lymph and CSF was always low so 100 minutes of counting time was used The vol ume of the perilymph or CSF samples was about 20 μl and the endolymph sample ranged from 1 0 to 1 5 μl Great care was used when taking the samples to prevent contamination with blood which had a high activity Blood sample volumes were 10 μl in all cases All samples except those of the endolymph had enough activity so that the accuracy in count ing was within 5% In the endolymph samples the counting rates were only 2 to 10 cpm above a background of 10 to 11 cpm so that these values were only reliable within about 20% The counting accuracy could have been im proved by increasing the specific activity and adding more total activity The total activity was kept small in order to reduce any radia tion-induced artifacts Using the above proce dures the maximum whole body radiation dose to the guinea pigs for the duration of the ex periments was less than 10 rads

The stimulus-related cochlear pot were recorded from the basal and third t the cochlea with differential electrodes technique for recording the cochlear phonic (CM) and the whole-nerve actio tential (AP) has been fully described else (Konishi et al 1961) The tone bursts u acoustic stimuli were generated by a on condenser earphone and delivered to t ternal ear canal in a closed system The pressure level was measured near tympanic membrane by using a calib probe tube The input–output functions o and AP as well as the magnitude of th docochlear potential (EP) were measur both methyl mercury treated animals and trols

The technique of collection of the dolymph was essentially the same as th scribed elsewhere (Mendelsohn & Ko 1969) Smooth collection of the endol sample resulted in a change no greater th mV in EP Samples of the perilymph obtained with micropipettes The pipette inserted into the scala vestibuli of the turn and connected to a withdrawal p via a polyethylene tube filled with w equilibrated mineral oil The rate of withd al of the perilymph was 20 $\mu l/min$ The ples of CSF were obtained from the cist magna. The collection technique was es tially the same as that used for perilymph lection Extreme care was taken for avoi contamination of those samples with bl The samples were examined under a mi scope and if samples contained blood th samples were discarded The blood sam were obtained by vardiac puncture w periodic collection of blood samples necessary the superficial jugular vein cannulated with a polyethylene tube

RESULTS

Changes in cochlear potential

No clinical signs of mercury poisoning w observed in 6 guinea pigs exposed to met

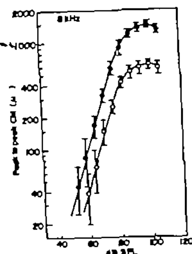


Fig. 1 Input-output function of cochlear microphonic (*CM* peak-to-peak voltage at 8 kHz measured with differential electrodes in the basal turn of the cochlea. The input-output function (O) (mean values) is obtained from 6 guinea pigs treated with daily administration of 2.4 mg Hg/kg of [14]C-labeled methyl mercury chloride for 7 days (total dose, 17 mg Hg/kg). The data were obtained one day after the last injection. The input-output function (●) mean values) obtained from 10 control guinea pigs is shown for comparison. Vertical range bars indicate standard deviations

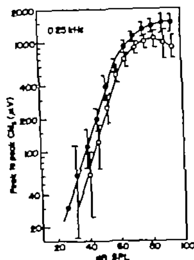


Fig. 2 Input-output function of cochlear microphonic in response to 0.25 kHz tone bursts recorded in the third turn of the cochlea. ● Control mean values O mean values obtained from the same group of guinea pigs treated with [14]C-labeled methyl mercury chloride as shown in Fig. 1

mercury by daily injections of [14]C-labeled methyl mercury chloride (2.4 mg Hg/kg) over 7 days (a total dose of 17 mg Hg/kg). When the cochlear potentials were recorded one day after the last treatment the peak-to-peak CM voltage in the basal turn of the cochlea in response to 8 kHz tone bursts was suppressed as shown in Fig. 1. Also shown for comparison are the mean values and standard deviations obtained from 10 control guinea pigs treated with Na_2CO_3. All guinea pigs treated with methyl mercury appeared more than one standard deviation less sensitive than control animals except for intensities lower than 70 dB SPL. The mean value of the maximum CM output was 0.5 mV compared with 1.4 mV in controls. The sensitivity of the CM expressed by the sound intensity necessary to elicit 100 μV peak-to-peak CM voltage was also reduced in animals treated with methyl mercury by 6 dB. Fig. shows the peak-to-peak CM voltage for 0.25 kHz tone bursts recorded in the third turn as a function of the sound intensity. The input-output function of the CM recorded from the third turn of the cochlea did not show deviations larger than one standard deviation from that obtained in control guinea pigs (Fig. 2). The input-output function of the CM in response to 0.25 kHz tone bursts recorded from the basal turn of the cochlea of guinea pigs treated with methyl mercury did not show a decrease of the maximum output of the CM but the sensitivity of the CM was reduced by 6 dB.

Striking suppression of the tone-evoked responses in methyl mercury treated guinea pigs was seen in the AP. Fig. 3 shows the amplitude of AP as a function of the sound intensity. For comparison the means and standard deviations in control guinea pigs are shown. The AP rose abruptly from a just detectable level of the AP and the hump around 60 dB SPL normally observed in control guinea pigs was not seen.

The mean EP recorded in the basal turn in guinea pigs treated with methyl mercury was 85.3 mV with a standard deviation of 3.2 mV. These values were comparable to those

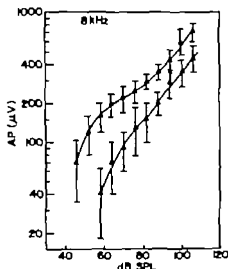


Fig 3 Whole-nerve action potentials are plotted as function of sound intensities of 8 kHz tone bursts. ▲ Control mean values Δ mean values obtained from the same group of ¹⁴C-labeled methyl mercury chloride treated guinea pigs as shown in Fig 1

obtained in control guinea pigs (83 8±4 2 mV) although the CM and AP were considerably suppressed in the basal turn of the cochlea.

Uptake of methyl mercury in the cochlear fluids

The kinetics of methyl mercury uptake and elimination were obtained in 8 guinea pigs treated with a single intravenous injection of 0.2 mg Hg/kg of ²⁰³Hg labeled methyl mercury chloride As shown in Fig 4 the mercury level in the whole blood dropped rapidly during the first day after injection when it was distributed throughout the body After the initial fast decline the blood level fell more slowly with a half life of about one week The concentration of mercury in the perilymph and CSF rose during the first day reaching a peak near the end of the first 24 hours After peak concentrations were reached the concentration in the perilymph endolymph and CSF appeared to diminish rapidly in the initial phase and then to fall slowly with a half life similar to that in whole blood of about one week

When the dose of a single injection of methyl mercury was increased to 1 7 mg Hg/kg in 8 guinea pigs the kinetics of mercury uptake and elimination were similar to those observed in guinea pigs treated with 0.2 mg Hg/kg of methyl mercury with the initial drop in blood concentration being slower (Fig 5). In 8 guinea pigs treated with a large dose of methyl mercury (17 mg Hg/kg) the mercury levels in the perilymph and CSF reached their peaks earlier than in guinea pigs treated with 0.2 or 1 7 mg Hg/kg The initial decline of the mercury concentrations in the whole blood was also slower than in those animals treated with 0 2 mg Hg/kg of methyl mercury The endolymph showed a peak concentration near the end of the first day The concentration of mercury after 10 days was approximately 10^{-2} μg/g in the perilymph and CSF (Fig 6)

The distribution of mercury was determined in 6 guinea pigs in which the ¹⁴C-labeled methyl mercury was injected intramuscularly each day for 7 days The total dose was 17 mg

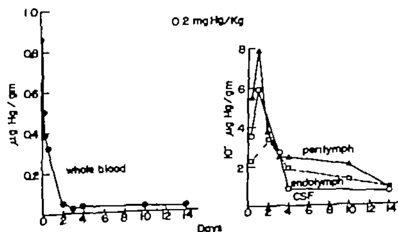


Fig 4 Total mercury in whole blood (left panel) and perilymph endolymph and cerebrospinal fluid (right panel) after intravenous injection of 0 mg Hg/kg of methyl mercury chloride labeled with ²⁰³Hg Each point represent single determination

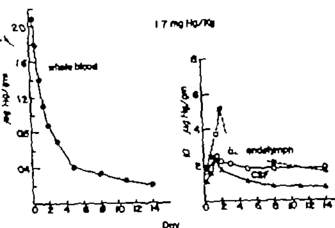


Fig. 3. Total mercury in whole blood (left panel) and in perilymph, endolymph and cerebrospinal fluid (right panel) after intravenous injection of 1.7 mg Hg/kg of methyl mercury chloride labeled with ^{203}Hg. Each point represents a single determination.

Hg/kg. One day after the last injection the concentration of mercury in the whole blood, perilymph, endolymph and CSF was respectively 7.12 μg/g, 2.89×10⁻² μg/g, 1.38×10⁻¹ μg/g and 2.64×10⁻ μg/g. These values were very nearly equal to those obtained in the intravenous injection of 17 mg Hg/kg after 7 days, indicating that the method of injection of methyl mercury was little effect on the resultant distribution.

Since methyl mercury chloride is broken down in the body, there is a question whether the concentration of methyl mercury observed in the samples is in the form of methyl mercury or of inorganic mercury. In order to examine this possibility a dual-labeled experiment was carried out in which ^{14}C-labeled methyl mercury and ^{203}Hg labeled methyl mercury were injected intravenously. The injection ratio of ^{203}Hg to ^{14}C was 0.5. *Ratios of 0.44* and 0.46 were observed in the whole blood and CSF respectively after 7 days, indicating that the mercury observed in the whole blood and CSF remained primarily in the form of methyl mercury.

DISCUSSION

Audiological studies demonstrated a hearing loss in the high frequency range in more than 80% of patients with methyl mercury poisoning at Minamata Bay in the 1950's (Kurland et al. 1960). Mizukoshi et al. (1975) reported that hearing impairment in the early stages of the intoxication is caused by cochlear lesions but the severe hearing disturbance and loss of

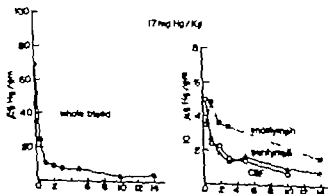


Fig. 4. Total mercury in whole blood (left panel) and in perilymph, endolymph and cerebrospinal fluid (right panel) after intravenous injection of 17 mg Hg/kg of methyl mercury chloride labeled with ^{203}Hg. Each point represents single determination.

speech discrimination at the later stages are attributed to retrocochlear lesions Similar observations were made by Fujisaki et al (1971) and Nosaka et al (1970) Falk et al (1974) reported on the basis of their histological studies that acute methyl mercury intoxication in guinea pigs resulted in degeneration of the outer hair cells of the organ of Corti at the upper turns of the cochlea. Our data demonstrate that the CM in the basal turn and the AP are considerably suppressed by methyl mercury whereas the CM recorded in the third turn remained little changed These findings confirm the conclusion made by Falk et al (1974) that methyl mercury poisoning resulted in the dysfunction of hair cells but the reason for the apparent disagreement between CM findings and site of maximal morphologic damage was not determined Recently Anniko & Sarkady (1978) reported that administration of mercury chloride resulted in morphological changes in the sensory epithelium in the apical part of the guinea pig cochlea in the early stage of intoxication although there is evidence in the literature that clinical symptoms of poisoning by inorganic mercury are different from those elicited by short-chain alkyl mercury salts (methyl ethyl and propyl compounds) (Swensson & Ulfvarson 1968) The fact that the EP was not suppressed in magnitude by methyl mercury indicates that methyl mercury does not interfere with the electrogenic pump mechanism in the stria vascularis This is in agreement with morphological findings (Falk et al 1974) that the stria vascularis and spinal ligament are not affected by methyl mercury The present study demonstrates suppression of the AP in methyl mercury treated guinea pigs indicating effects of methyl mercury on the neural structures Abnormal myelin sheath has been reported in rats treated with methyl mercury (Herman et al 1973) Similar observations were made by Miyakawa et al (1970) Since methyl mercury has a strong affinity to the neural tissue possible degeneration of the central auditory system cannot be excluded However our findings suggests that the primary site of action of methyl mercury is peripheral specifically the sensory organ of the basal turn of the cochlea which results in a characteristic hearing loss in the high frequency range

There seems to be little doubt that after intravenous administration of methyl mercury the cochlear fluids and CSF do not actively concentrate mercury Stupp et al (1973) studied the kinetics of streptomycin and kanamycin and concluded the organ specificity of ototoxic antibiotics is a result of accumulation in the cochlear fluids These antibiotics reach the inner ear by passive filtration from the blood but slow elimination results in a rapid increase of their concentrations in the perilymph and endolymph The elimination of mercury in the endolymph and perilymph was comparable to that in CSF at low dose (0.? mg Hg/kg) The data (Figs 5 and 6) indicate a slight decrease in the rate of elimination with higher doses (1 7 and 17 mg Hg/kg) However there is no large increase in concentration as occurs in the case of the ototoxic antibiotics

The half-life of mercury in the whole blood in guinea pigs is comparable with that in rats reported by Norseth & Clarkson (1970) and Yoshino et al (1966) Norseth & Clarkson (1970) reported that mercury in the blood is almost totally bound in the red blood cells and the blood too-plasma ratio of about 300 does not change for 50 days after injection The blood plasma contains only 0.5% or less of mercury contained in the blood Most of the mercury is bound to protein (Hughes 1957 Berlin 1963) and there is very little free in the plasma water As the mercury concentration ratio of the blood relative of CSF or the cochlear fluids is greater than 200 1 it is most likely that the mercury level in the cochlear fluids and CSF is comparable to the mercury level in the plasma It has been reported by Hughes (1957) that the intracellular mercury content is higher than that of the extracellular medium Variations of the extracellular concentrations such as those which were observed in CSF perilymph and endolymph may have

little effect on the intracellular concentrations of mercury in the cochlear tissues The intracellular mercury content may be determined by the number of binding sites available for the tissue (Hughes, 1957 Passow et al 1961) Thus may account for suppression of the CM and AP by methyl mervury even though the concentration in the extracellular fluids is small

Biotransformation of methyl mercury has been studied in rat by Norseth & Clarkson 1970 They reported that small amounts of inorganic mercury was detectable in plasma, brain and tissues involved in excretion Our experiments with dual-labeled methyl mercury indicated that the mercury in CSF and whole blood is in the form of organic mercury Therefore it is likely that the methyl mercury in the cochlear fluids is not biotransformed to any great extent

ZUSAMMENFASSUNG

Meerschweinchen bekamen sieben Tage lang tägliche subkutane Verabreichungen von ^{14}C-markiertem Methylquecksilberchlorid dessen Gesamtdosis 17 mg Hg/kg betrug In diesen Tieren wurden die Cochleamikrophone und die Gehörnerv-Wirkungspotentiale in der Basalwindung unterdrückt, aber es konnten keine wesentlichen Verluste in der dritten Cochleawindung registriert werden Die Stärke des Endocochlearpotentials wurde nicht beeinträchtigt Am Behandlungsende gab es keine Quecksilberanhäufung in der Perilymphe der Endolymphe oder in der Zerebrospinalflüssigkeit Quecksilberaufnahme und -ausscheidung in den Cochleaflüssigkeiten wurde in Meerschweinchen untersucht, denen eine Einzeldosis von mit ^{203}Hg markiertem Methylquecksilber [illegible] injiziert wurde, die zwischen 0,[illegible] mg Hg/kg und 17 mg Hg/kg lag. Die Befunde zeigten daß das Quecksilberkonzentrationsverhältnis im Blute den Cochleaflüssigkeiten gegenüber mit dem [illegible] berichteten Blut-Plasmaverhältnis vergleichbar [illegible] Im Gegensatz zu dem Fehlen einer Konzentration im Extrazellulärraum dürften die sensorischen Endorgane der Cochlea Methylquecksilber angesammelt haben

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EXTRACORPOREAL PRESERVATION

Organ Culture of the Post Natal Mammalian Inner Ear

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Abstract Postnatal (newborn and mature) inner ear organs from CBA/CBA mouse and guinea pig were analysed concerning hair cell survival in an in vitro sys. After only few days in the artificial surround- , transformation of hair cell characteristics occurred, he form of either loss of sensory hairs (cochlear hair s) or hair fusion (vestibular hair cells) although the cell itself survived for considerably longer time acellular myelin figures became evident after 2 3 s in culture. However considerable individual ation among hair cells was observed concerning the astructure of the cell at this stage in vitro Completely mal hair cells could in rare cases be recognized after week in organ culture (cochlear inner hair cells of the IA/CBA mouse) Loss of the surface structures of r hair cells is likely to constitute an irreversible trans mation not in agreement with the true hair cell char teristics

he introduction of new methods in the field f scientific investigation offers prospects for he further extension of our knowledge Re earch concerning the inner ear has frequently ocused interest on developmental biology he normal structure and function of the abyrinthine organs and problems connected with various fields of ototoxicity As the uman inner ear is such a complex organ he objective of animal experimentation is to ind model systems whose principles can also e applied in man.

Animal investigations performed *in vivo* can in part be undertaken even *in vitro* which in general offers more controlled conditions The technique for *in vitro* studies on the inner ear was introduced by Friedmann (review 1965) and Van De Water & Ruben (1971) and allowed a detailed analysis of embryogenesis and cytodifferentiation of the mammalian inner ear comparable to human conditions (Van De Water et al 1977 Anniko et al 1979 Nordemar & Anniko 1979)

In ototoxicity studies differences frequently occur between species and also between individual animals for a variety of reasons resorption after administration distribution in body compartments serum levels kidney function etc Friedmann & Bird (1961) described ototoxic damage to embryonic fowl *hair cells following streptomycin administration* However the situation in mammals can differ Sensitivity to ototoxic drugs is also likely to differ between developing embryonic sensory cells and fully mature physiologically functioning hair cells Whether or not the *in vitro* system can be applied in say ototoxicity testing requires further analysis describing not only the possibilities but also the limitations of extracorporeal preservation of the inner ear Embryonic and postnatal tissue have to be evaluated separately in such conditions.

The fundamental principle in an *in vitro* system is to obtain standardized and well *controlled conditions comparable to those in vivo* enabling the study of strictly defined

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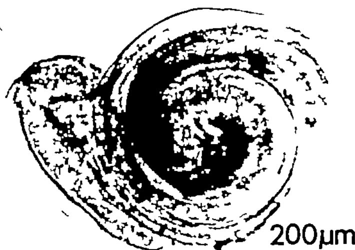


Fig 1 Light microscopy (LM) Explant of the cochlea *in toto* from the newborn CBA/CBA mouse cultured days in vitro

myelin figures increased in number intracellularly Explantation of single coils revealed well preserved cochlear hair cells during the first 1–3 days whereafter a great variation in hair cell survival became apparent. Degenerating and ultrastructurally quite normal hair cells could be observed adjacent to each other In general inner hair cells were less vulnerable than outer hair cells during the early stages of *in vitro* culture (Figs 4 and 5)

2. *Crista ampullaris* The general configuration of the organ was maintained during the entire observation period The shape of both types of hair cells and sensory hairs appeared normal during the first day in culture On the second day *in vitro* a small number of fusions of stereocilia became apparent, while most sensory hairs still displayed a preserved ultrastructure (Fig. 6) It was no longer possible to determine types of hair cells Cell junctions

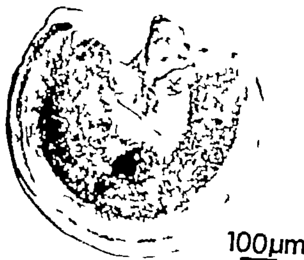


Fig 2 LM. Approximately half coil from mature guinea pig cochlea. The stria vascularis and Reissner membrane have been removed. Cultured *in vitro* for days.

parameters The hair cells comprise only one cell layer facing the endolymph and are to a a great extent surrounded by a water phase *even during normal conditions* receiving oxygen and nutrients largely by diffusion via the endolymph The present study was intended to determine whether or not the post natal inner ear can be adapted to *in vitro* conditions

MATERIAL AND METHODS

In the literature the CBA/CBA mouse is most frequently used in developmental investigations and it was therefore chosen for the present study so as to provide optimal reference material The guinea pig has been extensively studied with regard to ototoxicity investigations The inner ear organs from this animal were therefore analysed concerning survival after explanation of mature inner ears

CBA/CBA mouse

Inner ears of newborn and 2 month-old CBA/CBA mice were explanted for comparative study to an *in vitro* system consisting of Neuman and Tytell's (N & T) serumless medium freshly supplemented with 10% fetal calf serum (FCS) and 1% l-glutamine The inner ear organs were dissected free from surrounding cartilage and/or bone and explanted individually The cochlea was preserved either *in toto* or dissected into individual turns (Fig 1) The vestibular organs except for the utriculus were kept within their ampullar enlargements

Guinea pig (pigmented)

The animals used weighed approx 300 g Following decapitation the labyrinth was dissected in Hanks BSS (balanced salt solution) and all explanted organs were placed in the culture medium within 3 min The medium consisted of Neuman and Tytell's serumless medium supplemented with 20% guinea pig serum and 1% l-glutamine The cochleae were either cultured *in toto* preserving the tissues in the outlines of the endo- and perilymphatic spaces after removing the surrounding bone or else individual half-coils were placed in the medium after removal of the stria vascularis and Reissner s membrane (Fig 2)

General culture technique

The specimens were preserved at maximal air humidity at a temperature of +37°±0.2°C The medium was changed every second day and did not contain antibiotics/antimycotics. The gas phase consisted of normal air

Morphological documentation

After 1–7 days *in vitro* the specimens were fixed with 3% glutaraldehyde in 0 133 M sodium phosphate buffer post fixed in 2% osmic tetroxide and after dehydration in alcohol embedded in Epon Sections for light microscopy were stained with toluidine blue and thin sections for electron microscopy with uranyl acetate and lead citrate A Philips 400 microscope was used for ultrastructural studies

RESULTS

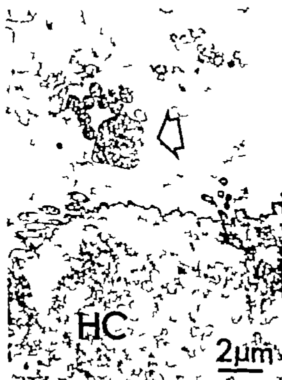
Hair cells were observed during the first week of *in vitro* preservation with special regard to early changes of the hair cell characteristics/ultrastructure

CBA/CBA Mouse

Newborn animal

I *Cochlea* In cochleae explanted *in toto* and containing all structures comprising the membranous labyrinth surviving inner and outer hair cells were observed after one week However following explantation nerve endings disintegrated during the first day On the second day loss of sensory hairs started but without hair fusion Even when hair cells had lost their sensory hairs the rootlets were still observed penetrating into the cuticular region (Fig 3) Cell organelles showed a normal ultrastructure but on the third day *in vitro*


1µm


HC
2µm


1µm


2µm



Fig 4 LM Disintegrating tiss the outer hair cell region from a cochlea explanted as a half coil (newborn CBA/CBA mouse) Se section of a specimen cultured in vitro for 4 days.

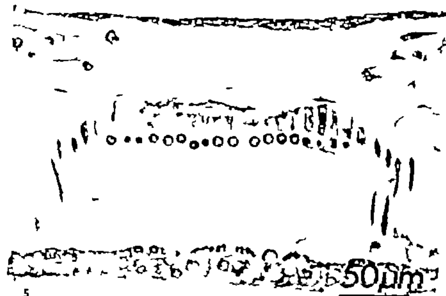


Fig 5 LM Serial section from the same specimen as in Fig 4 showi inner hair cells in place

and cell organelles were ultrastructurally unchanged A short stubby kinocilium frequently occurred on supporting cells as also thin microvilli irregularly arranged on the surface facing the endolymphatic partition

Following 3 days in culture a few myelin figures were observed in the hair cell cytoplasm (Fig 7) Only occasionally was the *cytoplasm of some hair cells more vesiculated* than that of surrounding hair cells indicative of an early stage of vesicular degeneration

As time went on *in vitro* fusion of either stereocilia alone or also including the kinocilium became more frequent as also did the formation of myelin figures intracellularly

Fig 3 Electron micrograph (EM) Outer hair cell fro the newborn CBA/CBA mouse cochlea cultured in vitro f 4 days Sensory hairs are lost but their rootlets pene trating into the cuticle are still identifiable Mitochondri are rather unaffected by the *in vitro* conditions Rough endoplasmic reticulum (ER) is found in the cytoplasm

Fig 6 EM Hair cell (*HC*) of the crista ampullaris fro newborn CBA/CBA mouse cultured days in vitro revealing normal morphology A part of the neighbour ing sensory hair bundle show hair fusion (*arrow*)

Fig 7 EM Detail from the cytoplasm of vestibular hair cell from mature CBA/CBA mouse A minimal myelin *figure is observed (arrow)* as also some vacuolization Cultured *in vitro* for 4 day

Fig 8 EM Preserved outer hair cell from guinea pig cochlea cultured in vitro for 3 day

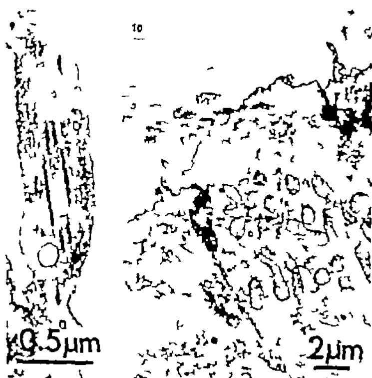
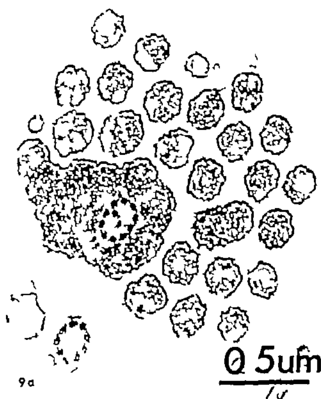


Fig. 10. EM. Hair cell from the mature guinea pig crista ampullaris after 5 days in culture showing normal ultrastructure.



Fig. 11. EM. Hair cell region in the mature guinea pig crista ampullaris after 1 week in culture. In some specimens widespread vacuolar type of degeneration occurred with formation of dark osmiophilic structures resembling accumulation of degenerating mitochondria (arrow).

tution giant hairs were observed (Fig. 9C). In these pathologic giant hairs one or several stereocilia could be identified but there also occurred a regular arrangement of microtubules/microfilaments in its cytoplasmic portion parallel with the enclosed sensory hairs and continuing into the inner parts of the hair cell. Adjacent hair cells could however still reveal preserved sensory hairs (Fig. 10). Intracellularly many mitochondria were ultrastructurally intact but other mitochondria were clumped together indicative of a degenera-



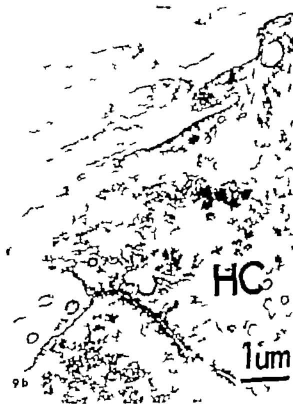


Fig 9 EM Crista ampullaris from the guinea pig (A) Sensory hair fusion including the kinocilium after 3 days *in vitro* (B) The sensory hairs are in part incorporated with the upper part of the hair cell (*HC*) cytoplasm while others still reveal a normal structure Cultured *in vitro* for 4 days (C) After 3 days or sometimes afte longer period of *in vitro* culture giant hairs had occasi ally formed indicating a fusion of several adjacent ster cilia.

Mature animal

The structural findings were similar to cells observed in inner ear organs of the newborn and no great difference in the reaction to the artificial environment occurred

Guinea Pig

Labyrinths from only adult animals were explanted A difference in vulnerability occurred between the vestibular and cochlear partitions of the inner ear

Cochlea

During dissection the bony walls of the cochlea were removed leaving the membranous structures intact However the stria vascularis was frequently falling over the organ of Corti

Nerve endings disintegrated during the first day in culture On the second and third days *in vitro* both outer and inner hair cells could show a vesicular type of cell degeneration On the third and fourth days after explantation many inner hair cells were still structurally preserved including sensory hairs while outer hair cells had frequently lost their structural organization and showed vesicular degeneration (Fig 8) Other inner hair cells were devoid of sensory hairs Hair fusion did not occur but myelin figures had often formed intracellularly A considerable variability subsequently became apparent in hair cell adjustment to the *in vitro* conditions—stability was lost in the *in vitro* system

Crista ampullaris

After 2 days in culture extracellular spaces were evident in the general structure of the cristae ampullares Sensory hair fusions started on the second day *in vitro* (Fig 9A and B) Already on the third day after explan-

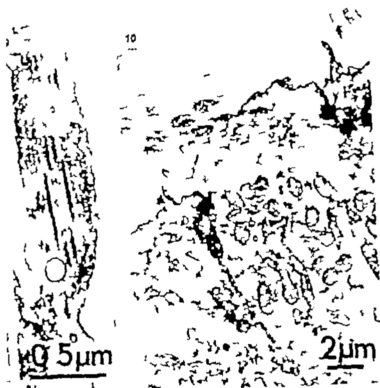


Fig 10 EM Hair cell from the mature guinea pig crista ampullaris after 5 day in culture showing normal ultrastructure



Fig 11 EM Hair cell region in the mature guinea pig crista ampullari after 1 week in culture. In some specimens widespread vesicular type of degeneration occurred with formation of dark osmiophilic structures resembling accumulation of degenerating mitochondria (arrow)

ation giant hairs were observed (Fig 9C) In these pathologic giant hairs one or several stereocilia could be identified but there also occurred a regular arrangement of microtubules/microfilaments in its cytoplasmic portion parallel with the enclosed sensory hairs and continuing into the inner parts of the hair cell. Adjacent hair cells could however still reveal preserved sensory hairs (Fig 10) Intracellularly many mitochondria were ultrastructurally intact but other mitochondria were clumped together indicative of a degener

tive process (Fig 11) Vesicular degeneration of a few hair cells and cytoplasmic myelin figures was observed In rare cases remnants of nerve chalices around vestibular hair cells of type I could be identified thereby determining type of hair cell After 5 days in culture a considerable morphological variation occurred concerning cell survival among individual hair cells though the majority of hair cells were present—although ultrastructurally changed—after 1 week *in vitro*

DISCUSSION

There is little published information defining the *in vitro* conditions of the differentiated mammalian inner ear Sobkowicz et al (1975) described preservation of hair cells and development of the innervation pattern in the organ of Corti of newborn mouse up to 27 days in culture but disorganization frequently occurred both among hair cells and in other parts of the organ of Corti after 2 weeks *in vitro* According to Weibel (1957) the organ of Corti in mouse is still immature at birth even in its basal turn and requires about 13 days of post natal development to become fully differentiated The degenerative process would thus start when explanted to *in vitro* conditions once a maturation is completed either obtained *in vivo* or *in vitro* Okano et al (1975) reported hair cell degeneration (phase contrast microscopy) after a few days *in vitro* concerning the cochlea of the newborn puppy

Yamashita & Vosteen (1975) maintained cochlear hair cells of the newborn guinea pig for more than 20 days *in vitro* when the organ of Corti was cultured *in toto* (light microscopic observation of the living explant) They also suggested that hair cells cannot develop (regenerate) *in vitro* a fact analysed by Ruben as early as 1967 describing how cells differentiating into hair cells pass their terminal mitosis before such a maturation occurs

Previous studies have described the preservation of hair cells without regard to early subtle ultrastructural changes in the hair cells

Anniko & Van De Water (1978) were succ ful in culturing the newborn mouse c ampullaris for one week but hair cells c not be identified with certainty even at ultrastructural level

Alterations in the hair cell fine struc is the first step towards a transformatio the hair cell characteristics no longer in a ment with the structure and function of highly specialized neuroepithelial cell sory hair loss (cochlear hair cells) or fu (vestibular hair cells) is probably beyond plete recovery This alteration must inte with the physiological function of the thereby changing its characteristics to a primitive type of cell and only preserving basal metabolism when adjusting it to *in vitro* environment

Injury to sensory hairs of the inner ear cells is an early feature in hair cell patho (Wersäll et al 1971 Hawkins 1977) ind ing that the neuroepithelial cells have their capacity to function as receptor for external impulses Also intracytopla myelin figures are frequently observed in ear pathology but this is probably not a definite injury to the hair cell as is loss of sensory hairs and might be overc if the toxic exposure is withdrawn befor hair cell passes the point of no return biochemical/physiological/structural da (reversible pathology/recovery)

The specialized surface structure of the cell can be obtained *in vitro* in embryo tissue (Anniko et al 1979) but is also the structure to be lost—except for degenera adjacent nerve terminals—when expla post natal inner ear organs to the *in* environment The hair cell itself may sur for one week or more but it is not likely this cell still possesses the characteristic a hair cell either physiologically or mor logically thus indicating an early irrever transformation which can be detected onl the ultrastructural level Also when a spe pathologic transformation pattern occur hair cells of explanted post natal inner

organs following exposure to toxic stimuli e.g. aminoglycoside antibiotics such an effect is difficult to establish in the present *in vitro* system

ZUSAMMENFASSUNG

Postnatale neugeborene und vollentwickelte Innenohrorgane von CBA/CBA-Mäusen und Meerschweinchen wurden das Überleben der Haarzellen betreffend in einem *in-vitro*-System analysiert. Nach nur einigen Tagen in der artifiziellen Umgebung geschah eine Transformation der Charakteristik der Haarzellen entweder mit Verlust der sensorischen Haare (cochleäre Haarzellen) oder einer Haarfusion (vestibuläre Haarzellen) obwohl die Haarzelle selbst bedeutend längere Zeit überlebte. Intrazelluläre Myelinfiguren wiesen sich nach 2–3 Tagen *in vitro*. Man merkte jedoch eine bedeutende individuelle Variation unter den Haarzellen die Ultrastruktur der Zelle in diesem Stadium betreffend. Vollständig normale Haarzellen konnten in seltenen Fällen nach 1 Woche in der Organkultur wahrgenommen werden (cochleäre innere Haarzellen der CBA/CBA-Maus). Ein Verlust der Oberflächenstruktur der Haarzellen wird wahrscheinlich eine unwiderrufliche Transformation, die nicht mit der wirklichen Charakteristik der Haarzellen übereinstimmt, ausmachen.

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PULSATILE TINNITUS AND THERAPEUTIC EMBOLIZATION

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Abstract Fifteen patients with disabling pulse synchronous tinnitus were investigated with super-selective angiography demonstrating an arteriovenous malformation in 8 cases chemodectoma of the jugular bulb in 3 and a local arterial stenosis in one case In 12 of these cases the murmur could be registered objectively while in the 3 cases with a negative angiographic finding no such murmur could be heard an observation which may be of importance when selecting patients for further angiographic examination. The cases with a tumour and those with an arteriovenous malformation were all treated with gelatin sponge embolization The immediate effect on the tinnitus was good in all cases and lasted more than months in 75% of the cases. No serious side effects were registered In selected cases embolization is recommended alone or in combination with surgery

Pulsatile tinnitus is a rarely occurring symptom of vascular origin When intense and of long duration the bruit may be agonizing to such a degree as to cause social disability and to provoke severe depressive reactions necessitating active therapeutic measures Encouraged by the experiences of mainly French radiologists (Djindjian et al 1973 Manelfe et al 1974) we have over the last 5 years or so applied therapeutic intra arterial embolization in selected cases This paper deals with our experiences concerning the selection of patients deemed suitable for this form of therapy and our assessment of the therapeutic results

CLINICAL MATERIAL

The material consisted of all 15 patients examined since 1972 with selective angiography because of severe pulsatile tinnitus The examination gave positive diagnostic information in 12 cases revealing a tumour of the jugular glomus in 3 cases an arteriovenous malformation in 8 and a stenosis of the external carotid artery in one (Table I) All these 12 patients also exhibited other objective signs In the 3 patients with a tumour this could be observed through the ear drum and in the remaining 9 patients with vascular disorders there was an audible murmur to be heard at auscultation In the 3 cases in which a murmur could not be registered extensive angiography was negative demonstrating no arteriovenous malformation and no signs of tumour

TECHNIQUE

Routine angiographic techniques were used with transfemoral or transcarotid catheterization The catheters used varied in inner diameter from 1 10 to 1 40 mm and in outer diameter from 1.57 to 1 90 mm As contrast medium Isopaque Cerebral 60% (Nyegaard) was used in the first 10 cases Amipaque (Nyegaard) in the last 5

For embolization absorbable gelatin sponge (Spongostan Ferrosan AB) was used cut into thin strips measuring about 1×1×8 mm The strips were placed one at a time into the tip of a syringe filled with saline Contrast medium was injected immediately before and after each embolization and followed by fluoroscopy in order to check the position of the catheter tip and the result The number of emboli needed for occlusion of the feeding vessels varied from one to more than 70 depending on the size of the lesion and the size of the vessel The final result of the treat

Table I *Treatment and results in patients with positive angiographic findings*

Case	Age Sex	Diagnosis	Arterial supply	Embolization	Results, immediat	Results, late
1 A A.	♀ 67	Spontan. carotid--cavernous fistula	eca dx (ama, ma, apha) +++ eca sin ++ ica dx + ica sin +	1) ma dx 2) ma dx (2 m later) 3) ma sin (1 w later)	Arterial supply eliminated Tinnitus eliminated	2 years later still good effect on vasc. No tinnitus
H B	♂ 25	Right-sided retroauric. malform.	oa dx ligated ma dx +++ apha ++	1) eca dx 2) ma dx (2 m later) 3) apha dx, ma dx (5 years later)	Good effect on vasc. Tinnitus reduced	*Slightly reduced* vasc. after 5 years Tinnitus returned to same severity in 1 month after all three treatments
1 N	♂ 71	Left-sided retroauric. a. malform.	oa sin +++ ma sin ++	oa sin	Good effect on vasc. Tinnitus eliminated	Slight effect on vasc. 1 month later. Bruit still audible but reduced and tolerable 3 years after treatment
H L	♂ 68	Left-sided retroauric. malform.	oa sin +++ ma sin +++	1) eca sin 2) eca sin (2 m later)	*Good effect* on vasc. Tinnitus markedly reduced	Tinnitus still reduced after 3 years
P N	♀ 43	Left-sided retroauric. malform	mma sin +++ ma sin +++ oa sin ++ apha sin +	ma sin *oa sin*	Good effect *on vasc* Tinnitus eliminated	Tinnitus as before treatment after 2 months. Angiography reveals retrograde *filling* of ica from eca. Therefore no further embolization
M M	♀ 30	Left-sided meningeal malform	ma sin +++	*ma sin*	*Arterial* supply eliminated Tinnitus eliminated	After 6 months, no tinnitus
H N	♂ 70	Left-sided retroauric malform	ma sin ++ ica sin +	ma	Good effect *on vasc. supply* Tinnitus reduced	After months, still reduced and tolerable tinnitus
B E	♂ 37	Right-sided retroauric malform	oa dx +++ sta dx + ma dx ++ mma dx +	oa dx *sta dx* ma dx	Good effect on vasc supply Tinnitus eliminated	After 2 months, still no tinnitus
D P	♀ 64	Right-sided jugular glomus tumour	apha dx + + ma dx	1) apha dx 2) apha dx (3 m later)	Good effect on vasc supply. Tinnitus markedly reduced after each embolization	1 year after last treatment, still good effect on tinnitus
0 A P	♂ 35	Left-sided jugular glomus	apha sin + ma sin + ica sin +	1) apha sin ma sin 2) apha dx (2 w later)	Vascular supply almost eliminated. Tinnitus eliminated	1½ years after treatment, still no tinnitus
T N	♀ 67	Right-sided jugular glomus tumour	apha dx + + oa d + ica dx	apha dx oa d	Arterial supply almost eliminated Tinnitus eliminated	Not known
1 L	♀ 72	Stenosis of eca sin		*0 therapy*		

Abbreviations used are explained in Fig. 1. The arterial supply was classified as follows: ++ = major feeders, ++ = significant contribution to the arterial supply   minor contribution.

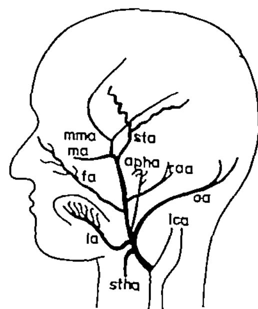


Fig 1 Major branches from the external carotid artery
Abbreviations
stha = superior thyroid artery
la = lingual artery
fa = facial artery
ma = maxillary artery
mma = middle meningeal artery
sta = superficial temporal artery
apha = ascending pharyngeal artery
raa = retroauricular artery
oa = occipital artery
eca = external carotid artery
ica = internal carotid artery

ment was always documented angiographically before withdrawal of the catheter from the external carotid artery

RESULTS

The results in the individual cases are presented in Table I Embolization was performed once in 6 patients twice in 3 and three times in 2 patients with varying intervals depending upon severity of persisting tinnitus or to recurrent symptoms In the tumour group the effect was persistent in 2 cases with a follow up for more than one year Of the 8 patients with an arteriovenous malformation the tinnitus returned to its original severity in only 2. A lasting satisfactory effect of the embolization was thus encountered in 75% of the patients treated

It should be pointed out that after treatment all of the patients experienced some pain attributable to the regions supplied by the embolized vessel The pain was most pronounced on the day following the treatment and was in isolated cases so marked that heavy sedation was necessary One patient with a jugular tumour (no 10) treated twice with embolization of the ascending pharyngeal artery on both sides developed a transitory facial palsy after each treatment Another patient with a left sided retroauricular arteriovenous malformation (no 3) developed aphasia after the treatment The symptoms disappeared during the subsequent 24 hours

DISCUSSION

The disorders causing pulsatile tinnitus may be of different types Holgate et al (1977) have described the symptom in connection with vascular tumours in the cerebello-pontine angle Neumann & Grossgerge (1977) have reported on a high-positioned jugular bulb or an internal carotid artery without bony covering towards the middle ear as a probable explanation Most frequently however the symptoms are due to an arteriovenous malformation to a tumour of the jugular glomus or to a local arterial stenosis as in our cases

The audible sound may result from turbulence in the blood stream secondary to a change in the blood's velocity as it passes through an arteriovenous fistula or a highly vascularized tumour of the jugular glomus. In cases of local vascular stenosis the intraluminal irregularity itself might be responsible for the turbulence In all of our patients with an arteriovenous fistula symptoms occurred spontaneously in other materials skull fracture has been described in connection with the development of the fistulas (Stoll & Kühner 1975) The three jugular tumours in our series were all considered inoperable The embolization in these cases was not intended to have a curative effect but was performed in order to give relief to the patient On the other hand



Fig. 3 a. Spontaneous carotid-cavernous fistula (case 1). Lateral projection.

t is known (Hekster et al. 1973) that operation may be facilitated by preoperative embolization and this is our definite experience with tumours in the facial region.

In our cases with an arteriovenous malformation, operation was thought to be of no or limited value mostly due to its extensive blood supply and/or to its extensive size. In some cases, on the other hand, embolization of small vessels close to the nidus of the arteriovenous malformation was considered to be a more appropriate measure than operation with ligation of larger, more centrally localized vessel.

In order to obtain the best possible results a thorough charting of the arterial supply should be performed prior to embolization. This includes selective angiography of all the major vessels that can possibly be involved in supplying a tumour—or an arteriovenous malformation. After such a charting the vessels suitable for superselective catheterization can be chosen and properly embolized. It should be stressed that the new non-ionic contrast agent, Amipaque, is a definite improvement on earlier used media, since scarcely any discomfort is experienced by the patient.

The method of embolizing vessels supplying an arteriovenous malformation is well documented (Newton & Adams 1968, Djindjian et al. 1973, Manelfe et al. 1974) and since a surgical approach often requires a rather extensive procedure in order to be effective (Hugosson & Bergström, 1974) embolization is well worth trying. In elderly patients and in patients who decline surgery, it is the sole alternative. In this context it should be pointed out that some malformations, such as spontaneously occurring carotid-cavernous fistulas seem to have a great tendency to spontaneous occlusion secondary to thrombosis, sometimes evidently initiated by small changes in

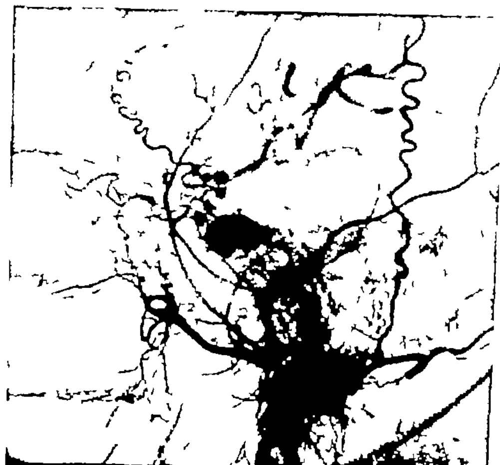


Fig 2b The same case (no 1) 2 years after embolization

the blood flow through the fistula (Brismar & Brismar 1976)

The aim of an embolization need not necessarily be a complete occlusion of all feeding arteries A decrease in blood flow with a reduction in the intensity of the tinnitus as a result is often enough Such an intentional *limitation of possible therapeutic results* is motivated as it decreases the evident risk involved in embolization This risk is connected with the possibility of a spillover of embolization material from the external artery to the internal carotid artery Evidently this occurred in one of our cases referred to above (no 3) though without lasting effects To avoid such a displacement of foreign material the tip of the catheter should always be as far peripheral as possible with one or more major branches from the external carotid artery central to the tip Any displaced embolus will then pass into one of these branches and not reach the internal carotid artery

Due to the risk involved embolization should be regarded as a surgical procedure and the patient must consequently by adequately and extensively informed before treatment In view of the limited number of patients for whom embolization is a conceivable therapeutic alternative it is preferable that this form of treatment *should be concentrated* on a limited number of clinics

ZUSAMMENFASSUNG

Fünfzehn Patienten mit schwerer Behinderung durch pulssynchrones Ohrensausen sind mit superselektiver Angiographie untersucht worden Die Befunde deuteten in acht Fällen auf arteriovenöse Mißbildungen, in drei Fällen auf Chemodektome des Bulbus V jugularis und in einem Fall auf eine lokalisierte arterielle Stenose In zwölf dieser Fälle konnten die Pulsationen bei der Untersuchung belegt werden. In drei Fällen mit negativem angiographischem Befund konnten keine Pulsationsgeräusche auskultiert werden Für die Auswahl von Patienten zur fortgesetzten angiographischen Untersuchung wird eine Auskultation als zweckmäßig erachtet Sämtliche Fälle mit Tumor oder arteriovenöser Mißbildung wurden mit Embolisierung durch Gelatineschwämme behandelt Der unmittelbare



Fig 3 Right-sided jugular glomus tumour Internal carotid angiography (case 9). Lateral projection.



Fig 3 b Case 9 Selective angiography of right ascending pharyngeal artery which in this case is a branch on the internal carotid artery

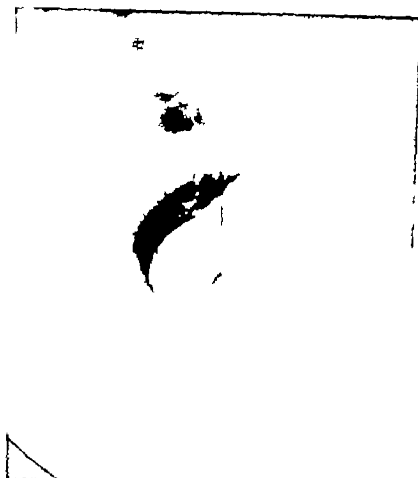


Fig 3c Case 9 Selective angiography of rigth ascending pharyngeal artery 2 months after embolization. Lateral projection.

Effekt auf das Ohrensausen war in sämtlichen Fällen gut und in 75 % der Fälle von mehr als zweimonatiger Dauer Keinerlei ernsthafte Nebenerscheinungen wurden bemerkt In ausgewählten Fällen wird eine Embolisierung als einzige Behandlung oder in Verbindung mit einem chirurgischen Eingriff empfohlen.

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VESTIBULAR RECRUITMENT AND DECRUITMENT

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Abstract Serial Thermal Vestibulometry with the application of increasing caloric stimuli (corresponding to decreasing temperature of water at successive irrigation of the ears) was performed on normal subjects and patients with lesions in the central nervous system involving the statoacoustic subsystem. The normal values and slope values (i e. increase in degrees of velocity of slow component of nystagmus in response to each 4°C change in water temperature) were computed to yield values of the normal vestibulograms. The values obtained in patients were compared with those of the Normograms and the following diagnostic patterns observed (*a*) Hypograms, (*b*) Hypergrams, (*c*) Central Vestibular Recruitment, and (*d*) Central Vestibular Decruitment. Central Vestibular Recruitment has been explained on the basis of the locus of the lesion leading to involvement of the nystagmogenic area in the lower part of the reticular formation, suprasegmental neurones in the vicinity of the vestibular nuclear complex and partial destruction of the complex itself each being responsible alone or in combination. Hypergrams presumably result from suprasegmental lesions, e g in cerebrum, cerebellum and stem components. Central decruitment follows neural lesions in the vestibular subsystem Hypograms are found in vestibular nuclear pathology Furthermore it helps in charting plan for therapy viz head and balance exercises, in particular in the sense that this exercise is not helpful in cases with central decruitment but is in peripheral one An explanation for this has been put forward

'Recruitment' with regard to the auditory system is an established phenomenon. It is expected that the vestibular component of the statoacoustic subsystem as is very rightly termed by Hinchcliffe may also show an identical phenomenon. The term recruitment was originally used by the neurologists Recruitment is used to describe the phenomenon whereby many reflexes either excitatory or inhibitory in nature gradually increase to a maximum as a stimulus is prolonged even though its intensity remains unaltered" and the phenomenon reflects activation of an increasing number of excitatory or inhibitory neurones (Eisenberg, 1958) In fact, it implies an outburst of response as a function of summation to a series of individual stimuli

Litton & McCabe (1966) described 'Thermal Vestibulometry' and demonstrated a normal hypofunctioning curve e g. in Meniere's disease and a descending curve as the stimulus is gradually increased indicating the presence of vestibular decay (cf auditory decay) in central nervous system lesions They failed to demonstrate vestibular recruitment though they had postulated it theoretically van Egmond et al (1949) observed a recruitment like phenomenon in cupulometry Albano (1957) and Horak (1962) also observed this phenomenon Torok (1970) noted both vestibular recruitment and decruitment. He is of the opinion that sensory or loudness decruitment is not identical with neural adaptation but vestibular decruitment is somewhat similar to it. Steffen & Linthicum (1970) did not show vestibular recruitment in their continuous thermal vestibulometry Claussen (197?) demonstrated hyperactivity in the Major Butterflies in central nervous system lesions but this hyperactivity is not recruitment, since multiple stimuli were not applied. In this connection one may consider the difference be

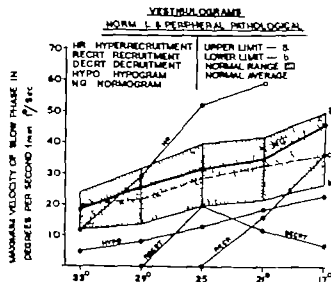


Fig 1 Normal and peripheral pathological vestibulogram

tween one type of hyperactivity e g phonophobia, and another oxyecoia as used in connection with the auditory system The former is hyperacusia observed at a level far above the threshold e g in paralysis of the stapedius muscle and the latter a real increase in the sensitivity of the endorgan observed at a slightly suprathreshold level which we refer to as recruitment Hyperactivity resulting from a lesion in the suprasegmental part of the central nervous system shows hyperactivity near the threshold and not in the sense of activating an increasing number of neurones consequent upon application of multiple stimuli

What is being described here we believe is true vestibular recruitment a real cumulative increase in the sensitivity/activity of the vestibular sub-system and decruitment implying neuronal lesion in response to gradually increasing stimuli We shall confine ourselves to the aspect of central' recruitment and decruitment the peripheral aspects can be found elsewhere (Ghosh et al 1976 Sarma et al 1977).

MATERIAL AND METHOD

Normal young subjects with no auditory or vestibular symptoms or signs were subjected to serial thermal vestibulometry the irrigating water temperature being 33° 29° 25° 21° and 17°C in increasing order with 4°C intervals between the successive stimuli The ear under test was irrigated for 40 seconds at each temperature with 5 minutes rest intervals. The subjects were in supine position with the head raised 30° above the horizontal level and the same set-up was used as described by Hallpike Cawthorne & Fitzgerald in their classical bithermal caloric test Electronystagmographic recordings were made through horizontal electrodes fixed at the outer canthi using a Cardiotrace 3050 The maximum velocity of the slow phase was calculated at each stimulus and the values plotted on a graph with the temperature on the abscissa and the maximum velocity on the ordinate resulting in the Thermal Vestibulogram (Fig. 1) Statistical evaluation was done and the maximum, minimum and mean values and the normal range of reaction was obtained (Fig 1) The accompanying table shows the values

This group is hereafter referred to as the control group and the vestibulogram resulting

Table I *Maximum velocity values (degrees/second) obtained by thermal vestibulography*

	33°C		29°C		25°C		21°C		17°C	
	Right	Left	R	L	R	L	R	L	R	L
Mean	19 18	20	4 0	26.45	32.27	31 0	35.82	34 64	41 09	41 45
S D	6 13	4.83	9 49	7.55	11 17	9 84	11 05	11.83	13 06	1_16
Range										
Maximum	_5.31	25 65	33 49	34 00	43 44	40.84	46.87	46 47	54 15	53 61
Minimum	13 05	15 99	14.51	18 90	21 10	1 16	4 77	22.81	28 03	29.29

Table II *The normal slope values*

	33–29°C	29–25°C	25–21°C	21–17°C
Range	1–7°/sec	2–10°/sec	3–4°/sec	3–7°/sec
Mean	4°/sec	6°/sec	3°/sec	5°/sec

from the values given as a normal thermal vestibulogram (Fig 1) Here the curve shows a gradually ascending pattern maintaining a fairly linear relation to the increase in stimulus strength Changes in degrees of the velocity of the slow phase per second per change of 4°C water temperature were calculated which constitute the slope values (Table II) Lastly the ears are irrigated with water at 45°C i.e 8°C above body temperature (to compare with the response at 29°C i.e. 8°C below body temperature) for studying the phenomenon of DP The following types of curves were obtained.

1 Normal (Fig. 1 NO)

2. Hypofunctioning (Fig. 1 HYPO) All values lie below the shaded area.

3 Hyperfunctioning or Hyperactive (Fig. 2 HA) The values lie above the shaded area.

4 Recruiting. (*a*) Peripheral (Fig 1 RECRT) Here the initial values are low but later these increase very rapidly as the stimuli are increased The curve does not maintain the normal slope values and consequently is not parallel with the normal curves (maximum minimum and mean) i.e at certain stimuli the slope values are higher than the normal ones Here the nystagmus often decreases on fixation (*b*) Central (Fig. 2CR) Here the initial values are higher than normal and fixation often increases the nystagmus. Subsequently the rise is steeper with increasingly higher slope values and the curve lies above the shaded area. In quite a number of cases this is followed by a decline in the curve De cruitment' (*vide infra*)

5 Hyperrecruitment (Fig. 1 HR) Here the initial values are low and there is a very steep rise in the curve reaching above the shaded area. Fixation reduces the nystagmus and thus is associated with peripheral lesions

6 Vestibular Decay or Decruitment. (*a*) Peripheral (Fig 1 DECRT) Here the initial values are normal or hypo subsequently the curve descends with successive stronger stimuli Fixation reduces the nystagmus in these instances (*b*) Central (Fig. 2, CD) Here the initial values are high (hyper) and then descend subsequently despite an increase in stimuli Fixation increases the nystagmus in this case This is thought to be due to neural involvement in the suprasegmental part of the vestibular subsystem.

OBSERVATIONS

(*a*) Peripheral patterns (hypogram peripheral recruitment hyperrecruitment and peripheral decruitment) have been discussed elsewhere in connection with the peripheral lesions (Ghosh et al. 1976 Sarma et al 1977) These patterns were noted in Meniere's disease streptomycin toxicity head injury syphilis (congenital) presbycusis (may rightly be termed Presbyatonia of Labyrinth') post-stapedectomy and juvenile diabetes mellitus

(*b*) Central patterns (i) Hypergrams (Fig 2, HA) (ii) Central recruitment (Fig. 2 CR) (iii) Central decruitment (Fig. 2 CD) These

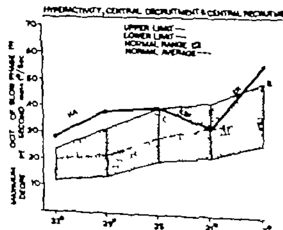


Fig 2 Hyperactivity central decruitment, and central recruitment

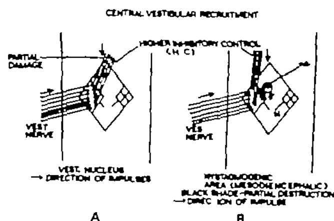


Fig 3 Central vestibular recruitment Mechanism of Higher Inhibitory Control (*HIC*) and effect of partial damage of efferent suprasegmental neurones (3 A) and nystagmogenic area (3 B-na)

curves are not hypothetical and have been derived from the values obtained in the actual ENG's of patients suffering from different central nervous system lesions These are the clear-cut typical varieties that we have come across But there is a continuum of a wide variety of curves showing atypical dispositions Lesions of the cerebral cortex (parietotemporal regions) brain stem and cerebellum showed hypergrams One case of parietotemporal region was associated with central recruitment Central recruitment was noted mostly in cases of brain stem lesion in the vicinity of the vestibular nuclear complex so also the phenomenon of central vestibular decruitment Frequently this was observed bilaterally as is usual with respect to auditory tone decay in brain stem lesions

DISCUSSIONS

In the conventional bithermal test only two temperatures are used This test may at times be fallacious since the patient may respond to stimuli outside these two Moreover distortion dissociation or reversion is usually evident when strong stimuli e g ice-cold water water at very low temperatures (e g at 21 C 17°C etc) are used so also the vegetative reactions (Ghosh & Sen 1970 Jadav et al 1971) Initial nystagmus (induced) may change on applying stronger stimulu using the same identical subnormal body temperatu indicating the presence of brain stem lesi which aspect cannot be assessed by the c ventional bithermal test (Ghosh & Sen 19 Weaker stimuli stimulate certain vestibu neurones which have been deranged by lesion with consequent altered reactivity stronger stimuli more and more neurones brought into action in a quaint way resulting the altered response referred to above. T perhaps is identical with the phenomenon central recruitment Reisco & Stroud (19 observed dysrhythmia in a case of medull blastoma of the vermis with bithermal test this disappeared when tested with strong stimulus (18°C) for 60 seconds Riesco-M Clure showed this in a proven lesion of roof and lateral walls of the fourth ventric In the bithermal test the phenomena of su mation and adaptation can not be ascertain whereas in serial vestibulometry it can be. it is a test of a much wider value than t bithermal one helping localize the lesion mo precisely It has been rightly pointed out Litton & McCabe (1966) that the bitherm test has the same disadvantage as looking a landscape down a long fixed and narrow tub

The present test induces ampullofugal d flection of the cupula in the horizontal sem circular duct bringing about depression in t

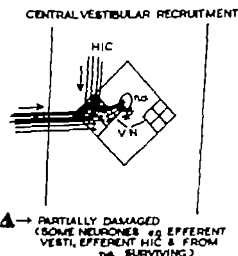
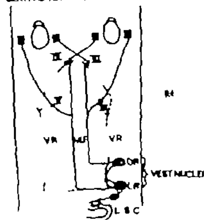


Fig 4 Central vestibular recruitment showing effect of partial destruction of vestibular nuclear complex.



SPIEGEL'S CONCEPT
DR - DEXTRO ROTATORY CELLS
LR LEVOROTATORY
MLF MEDIAL LONGITUDINAL FASCICULUS
R VESTIBULO RETICULAR PATH
L S C LATERAL SEMICIRCULAR DUC
● DAMAGED (TOTAL OR PAR IAL)

Fig 3 Depicts Spiegel's concept concerning relationship between ipsilateral and contralateral conjugate deviation via inhibition of antagonistic muscles.

resting potential in the vestibular nucleus complex with its obvious fallacies But multiple stimuli tend to minimise the above-mentioned fallacy and since identical stimuli (below body temperature) are applied in both the ears under the same controlled conditions the results are well acceptable

The hypofunctional curves were found in lesions in the peripheral vestibular apparatus up to the vestibular nuclei and as such the estibular nuclear complex is included in the peripheral vestibular component so far as the caloric induced nystagmography is concerned. Hyperfunctioning suggests a release phenomenon due to the partial/total withdrawal of the suprasegmental control from the vestibular nuclei (Ghosh & Sen, 1970) This hyperactivity is present starting from the initial stimuli with or without the presence of any sudden rise in the curve in response to gradually increasing stimuli (recruitment) or decruitment This was found in brain lesions (cerebrum, cerebellum and brainstem-high) post-head-injury etc Central vestibular recruitment is a very interesting and useful phenomenon observed in our studies If the initial values are higher than normal or high normal and increasing rapidly above the shaded area of the normal range we call it Central Vestibular Recruitment Here hyperactivity is associated with sudden increase in the response as the stimuli are increased i e all the values lie above the shaded area of normal range This was noted in cases of lesions in the brainstem and one case of an extensive space-occupying lesion in the temporo-parietal region. The possible mechanisms of central vestibular recruitment are as follows

1 Efferent suprasegmental neurones inhibit the vestibular nuclei When they are damaged largely or totally there would be hyper activity at all stimuli levels. But if they are damaged partially (Fig. 3 A) there would be hyperactivity initially If the stimuli are then increased in a graduated way the summated impulses consequent upon the increased neural activities would at a certain stimulus level surge above the level of the residual suprasegmental inhibitory control (offered by the surviving neurones) and would submerge it. These residual efferent neurones then fail to contain the very strong summated impulses after exerting the initial check on the weaker stimuli generated at the beginning of the test before the stimuli culminated into the strong summated effect A sudden response of high order follows accounting for the Central Recruitment' This was more evident when no appreciable time lag was allowed between the application of the successive stimuli thereby facilitating the generation of the summation effect Perhaps this is the cause of the fatigue which follows the attainment of the *maximum* summation effect and not infrequently following recruitment the decruitment *observed in* neural lesions This aspect warrants further *investigation*

2 Lachmann & Bergmann (*1961*) have shown that by stimulating the labyrinth the stimuli *arrive first at the* vestibular nuclei but nystagmus can appear only after the centre of

the reticular formation (i e nystagmogenic area) has been brought into action In a partial lesion in the nystagmogenic area or in the neuronal connection between this and the vestibular nuclear complex (Fig 3B) the nystagmus will be low upon low caloric stimuli during the initial stage due to activation of fewer neurones in the above mentioned areas There is often associated hyperactivity because of the suprasegmental neuronal involvement. With increasing stimulus more and more neurones of the nystagmogenic area are brought into action leading at a certain stage to a sudden increase in the response with heightened nystagmus accounting for the Central Vestibular Recruitment When there was no initial hyperactivity the effect of fixation on nystagmus helped to differentiate between peripheral and central lesions

3 Partial destruction of the vestibular nuclear complex (Fig 4) Initial stimuli would show diminution of the threshold response but stronger stimuli can excite a large number of neurones belonging to the vestibular–ocular–reticular system leading to Central Vestibular Recruitment Similar hypothesis may hold good in the case of peripheral recruitment resulting from epithelial lesion in the labyrinth Both would show identical curves in vestibulograph i e initial hyporeaction associated with recruitment But in suspected cases of brain lesion the nystagmus increases upon fixation since the nuclear complex is not completely destroyed and the suprasegmental influence is still retained to a certain extent This points to differing loci of pathology viz vestibular nuclear complex and the crista ampullaris under test but same modus operandi

4 According to Spiegel (1944) innervation of one longitudinal fasciculus in the ipsilateral conjugate deviation of the eyes is associated with inhibition of the antagonistic muscles producing conjugate deviation of the opposite side (Fig 5) This is brought about by the reciprocal mechanism through the internuncial connections between the levo- and dextrorotatory nuclear components in the vestibul nuclei Let us suppose that the LR ce (levorotatory cells) are partially damaged. Th DR cells will produce conjugate deviation o the eyes to the right with nystagmus to th left Simultaneously impulses will be sent t the LR cells from the DR cells through the in ternuncial connection inhibiting the tenden to conjugate deviation to the left and conse quently nystagmus to the right Now sinc the LR cells are damaged the inhibitory effe is ineffective and the nystagmus to the left i opposed to an extent by virtue of the antago nistic influence of the surviving neurones i the partially damaged LR cells acting un inhibited on the neural mechanism responsible for causing conjugate deviation to the left. S it follows that during the inital phase of stim ulation causing nystagmus to the left the nys tagmus cannot be strong because of the in hibition by the LR cells But under the impac of gradually increasing stimuli a stage is at tained when the DR cells will be very strongl stimulated sending their very strong inhibi tory impulses to the LR cells and thus sub merging the impulses spontaneously emanat ing from the surviving neurones in the par tially damaged LR cells which were for so long not inhibited by the DR cells. Consequently the tendency to produce conjugate deviation to the left is nullified Moreover the impulses sent by the DR cells will inhibit the LR cells (the surviving neurones) and thereby the conjugate deviation to the left is further depressed so also the occult nystagmus to the right The result is sudden uninhibited increase in the nystagmus to the left accounting for the Central Vestibular Recruitment Here too if suprasegmental neurones are involved one expects hypergram initially if not hypogram which can be unfolded by studying the effect of fixation on nystagmus

It appears that either of the above mechanisms—alone or in combination depending on the locus and extent of the pathology—is responsible for the phenomenon of Central Vestibular Recruitment

Vestibular Decay was present in two forms. (i) one with initial supranormal values which we think to be of central origin and (ii) the other with initial low (normal or subnormal) values which have been thought to be due to peripheral neural lesions (Ghosh et al 1976 Sarma et al 1977) This is perhaps associated with retrograde neural degeneration following endorgan lesion. This localization has clinical and therapeutic implication Though decay is quite often interchangeably used with habituation they are not the same phenomenon The former implies fatigue falling off of response or decline of response due to impairment of functional neuronal units under the impact of continuous or rapidly repeated stimuli whereas the latter implies the developing of a habit or getting used to it, i e a learning process under the above conditions For this learning process the reticular/centrencephalic system is the key mechanism which functions in conjunction with the vestibular subsystem when a patient undergoes head and balance exercises If there is central vestibular decruitment' the exercise is unlikely to offer any material help because the neural substrate necessary for habituation is at fault in central lesions. If on the other hand there is peripheral decruitment the exercise will help rehabilitate the patient since the above neural mechanism is available for the process. So it follows that during the exercise one should not prescribe the drugs which will suppress the above systems (e g. stemetil and other tranquillizers) and which thereby delay the learning process or render the systems non-responsive or ineffective Hence except during attacks of severe vertigo the tranquillizers should be withdrawn during the period the patient is undergoing head and balance exercises

Thus the serial thermal vestibulometry offers a visual graphic representation of the vestibular dynamics. One is apt to diagnose a case instantaneously on seeing the patterns of the Thermal Vestibulogram (comparable to audiogram) thereby obviating the tedious practice of going through the whole nystagmogram as has been suggested by Claussen (1972)

This is the preliminary report of our study A more intensive study is necessary and in progress in our laboratory in order to understand more about vestibular dynamics including recruitments decruitments hyper and hyposensitivities etc this has also given us a better insight into the nebulous subject of intermittent vertebrobasilar insufficiency (1977)

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Part of this work pertaining to the peripheral lesions has been accepted as thesis for the degree of M.S (E N T) conferred on Dr A Sarma (Sarma et al., 1977) W are thankful to Prof P N Tandon Head of the Dept. of Neurosurgery and his team for the facilities and for co-operation. We acknowledge our gratitude to Prof C. Claussen, President, Neurootological and Equilibriometric Society Reg, Wurzburg, West Germany for his valuable suggestions. W are also grateful to the Director and the Medical Superintendent of All India Institute of Medical Sciences, New Delhi, for the facilities and for permitting us to use the hospital records.

ZUSAMMENFASSUNG

„Serial thermal vestibulometry" mit der Anwendung von zunehmenden kalorischen Reizen (entsprechend abnehmender Wassertemperatur bei aufeinander folgender Spülung der Ohren) wurde durchgeführt an normalen Versuchspersonen und Patienten mit Verletzungen im zentralen Nervensystem, umfassend das statoakustische Untersystem Die normalen Werte und „Slope"-Werte (d h. Steigerung von Geschwindigkeitsgraden der langsamen Komponente des Nystagmus als Reaktion auf jede 4°C-veränderung der Wassertemperatur) wurden berechnet, um Werte des normalen Vestibulogrammes zu erhalten. Die von Patienten erhaltenen Werte wurden mit denen von Normogrammen erzielten verglichen dabei wurden die folgenden diagnostischen Muster beobachtet. a) Hypogramme, b) Hypergramme) zentrales vestibulares „Recruitment" und d) zentrales vestibulares „Decruitment" Zentrales vestibulares „Recruitment" ist auf der Basis der Verletzungsorten, erklärt was zu Einbeziehung der nystagmographischen Fläche im unteren Teil der retikularen Formation, suprasegmentalen Neuronen in der Umgebung des vestibularen nuklearen Komplexes und teilweiser Zerstörung des Komplexes als solchen führt, dabei ist jeder Faktor allein oder eine Kombination von Faktoren dafür verantwortlich Hypergramme entstehen wahrscheinlich aus suprasegmentalen Verletzungen, z.B in Cerebrum, Cerebellum und Stammkomponenten Zentrales „Decruitment" folgt auf neurale Verletzungen im vestibularen Untersystem. Hypogramme er den bei vestibularen nuklearer Pathologie vorgefunden. Dies hilft bei Ausarbeitung eines Planes für die Therapie

nämlich Kopf und Balanceübungen Insbesondere darum weil diese Ubung bei Fällen mit zentralen „Decruitment" nicht nützlich ist dahingegen bei peripheralem von Hilfe ist. Eine Erklärung dessen ist dargelegt worden.

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MODIFICATION OF THE MACAQUE'S VESTIBULO-OCULAR REFLEX AFTER ABLATION OF THE CEREBELLAR VERMIS

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Abstract I macaque the vestibulo-ocular reflex (VOR) as evaluated by the time constant of nystagmus in a modified Bárány spinning test, show regular pattern of change with time after ablation of the vermis cerebelli One day after ablation the time constant is in the low normal range it then increases, and after one week assumes values in the high normal range. While the time constant is high, the VOR is resistant to modification by repeated testing, but may be modified by unidirectional optokinetic nystagmus and by experience with reversing spectacles These results suggest that the vermis of the cerebellum plays no crucial role in modifications of the VOR by visual inputs, but is involved hen the VOR is modified by repeated vestibular experience.

Because it has anatomical and physiological relationships with other structures involved in eye movement, the vermis of the cerebellum has long been recognized as a *possible* important nexus in oculomotor function (Crosby et al 196_ Fuchs & Kornhuber 1969 Baker et al 1972 Ron & Robinson 1973 Gardner & Fuchs 1975 Llinás & Wolfe 1977) Aschoff & Cohen (1971) and Ritchie (1976) have described defects in visual-oculomotor behavior that attend upon lesions of the *vermal* cortex in monkeys In 1963 Singleton showed in the cat that damage to the vermal cortex interfered severely with acquisition and retention of habituation of nystagmus elicited by caloric stimulation Following this lead we have tested several stimuli to modification of the vestibulo-ocular reflex (VOR) in monkeys with total vermal ablations To sharpen the analysis we have used a rapidly performed and easily quantitated test of vestibulo-ocular function a modification of the B[illegible] spinning test. We have also evaluated our vermisectomized monkeys to determine what portion of the clinical oculomotor syndromes seen after complete cerebellectomy (Westheimer & Blair 1973) and hemicerebellectomy (Westheimer & Blair 1974) might be attributed to removal of the vermis alone

METHODS

These observations compare a large series of normal *Macaca speciosa* and *Macaca irus* with three *M speciosa* and one *M irus* which had complete cerebellar vermal ablations

To permit immobilization *of the head during* testing three stainless steel screws were implanted in the calvarium of each monkey During testing the monkey sat upright in a chair that could be rotated around a vertical axis *either at* a chosen constant velocity or in a *sinusoidal pattern* Performance was evaluated by direct observation and by analysis of vertical and horizontal electro-oculograms Electro-oculograms were recorded via non-polarizing skin electrodes and Grass P 18 amplifiers and were calibrated by repeatedly attracting the monkey's gaze to known points in the oculomotor field Time signal electro-oculograms chair movement and photoelectric indication of covering of the monkey's eyes were recorded on a Mingograf 800 Velocity of eye

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and chair movement was measured directly from the Mingograf records

The evaluations included 1) Spontaneous eye movements binocular conjugacy and convergence saccadic frequency dynamics amplitude and distribution over the oculomotor field gaze holding, and smooth pursuit. 2) Vestibular eye movements—the modified Bárány spinning test (B-test) The monkey in his chair with his eyes covered was accelerated around a vertical axis through a velocity step[1] usually of 100°/sec then held at constant velocity until his nystagmus subsided The B test was evaluated by plotting the velocity of the smooth phase of nystagmus as a function of time following the velocity step In the normal monkey this time course is approximately exponential so that its temporal aspect can be described by a single parameter the time constant i e the time required for the smooth phase velocity to drop to 37% of the value it has at any particular time In normal inexperienced *M speciosa* the average time constant is 24 7 sec with a range from 7 to 80 sec (values for 35 monkeys in their first B-test) Regression lines and statistics for B-tests were calculated on a logarithmic transformation of velocity measurements

The vestibulo-ocular reflex was modified by three procedures

(1) *Repeated B-testing* In the normal monkey repeated B-tests decrease the time constant of nystagmus in the B-test The decrease in time constant occurs slowly in monkeys who newly experience the B test and more rapidly (after 3–4 tests) in monkeys who have often experienced this modification The fully modified VOR shows a time constant of about 7 sec

(2) *Unidirectional optokinetic nystagmus* In the normal monkey unidirectional optokinetic nystagmus occasions an asymmetry in vestibular nystagmus (Tibbling 1970 Young & Henn 1973 Ornitz et al 1974) This asymmetry is manifest in the B-test as an increase in time constant of vestibular nystagmus in one direction (the direction of smooth phase optokinetic nystagmus) and/or a decrease the time constant of nystagmus in the op site direction The modification is achieved accelerating the monkey to a constant rota nal velocity of about 100°/sec waiting vestibular nystagmus subsides then uncov ing the monkey s eyes for a chosen period time so that he sees the laboratory envir ment apparently rotating around him. t elicits optokinetic nystagmus. The monke eyes are then covered and all optokinetic aft nystagmus allowed to subside Subsequent testing evaluates symmetry of the VOR.

(3) *Head-attached reversing-prism spe tacles* Head attached optical devices distu the usual stabilization of the image of vis space on the retina by the VOR they indu movement of the image on the retina dur head movement Such image movement quires modification of the VOR if stabilizati is again to be attained This modification the VOR does occur (Miles & Fuller 197 Gauthier & Robinson 1975 Gonshor & M ville Jones 1976) and is measured by sinu oidal rotation of the blindfolded monkey Th effects of optical devices may best be d scribed by the VOR gain that would be r quired if the monkey were to stabilize th image on the retina during head moveme while wearing the device Our monkeys wo and viewed their environment exclusive through a pair of Dove prisms bases plac bitemporally horizontal field of view 22° ve tical field of view 17° These devices wer worn while the monkey sat in his chair with h head free to move No special training wa provided but head movement was encourage by offering him a rich and changing visu environment

Vermal ablations were performed by suctio via a suboccipital incision and were intende to remove all vermal cortex and underlyin white matter and roof nuclei The extent o

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[1] Humane treatment of the monkey demand that accele ation be accomplished over a 1— second time period thus acceleration i not a true elocity tep.



Fig 1 Cerebellum of *M speciosa* after total ablation of the cerebellar vermis. The ablation included all of the fastigial nuclei and parts of the interposed nuclei, but spared the dentate nuclei.

vermal ablation was eventually determined by gross (Fig 1) and microscopic examination the dentate nuclei were spared the fastigial and varying portions of the interposed nuclei were removed In two monkeys there were small areas of intact residual vermal cortex We could find no correlation between variations in nuclear and cortical ablation and the slight variations in the subsequent vestibulo-oculomotor syndrome There was incidental to surgery damage to the left fourth nerve in one monkey causing transient weakness of infraduction and in-cyclorotation in the right eye

RESULTS

I Oculomotor and somatic motor status after vermisectomy

The vermisectomized monkey is fully conjugate shows no defect of pursuit or gaze holding, and converges normally He makes saccades of all normal sizes of normal dynamic characteristics and in all directions throughout the oculomotor field. Visual suppression of vestibular nystagmus is normal After surgery the frequency of spontaneous saccades increases significantly from a norm of about 1 75 saccades/sec to about 3 saccades/sec This polysaccadia changes little with time after surgery (Fig. 2) When the vermisectomized monkey is blindfolded his polysaccadia disappears (Fig 3) but when he is given a vestibular input while blindfolded his nystagmus has a higher frequency than normal Along with his polysaccadia, the vermisectomized monkey has a tendency to make short trains of repetitive alternating and nearly equal saccades a pattern which looks like a square wave on the *electro-oculogram* record Another abnormality perhaps related to the polysaccadia, is an apparent saccadic dys*metria*, manifested by a use of multiple saccades when the monkey attempts to fixate an object. Ritchie (1976) has investigated this dysmetria in detail in trained monkeys with vermal lesions All of our vermisectomized monkeys had the severe and lasting truncal ataxia characteristic in animals with vermal lesions

II Changes in time constant of nystagmus in the B-test after vermisectomy

After vermisectomy the time constant of nystagmus in the B-test shows a regular pattern of change In normal monkeys the time constant of nystagmus in the B-test may have any value from 7 to 80 sec when first tested but the time constant falls to an unchanging 6–8 sec

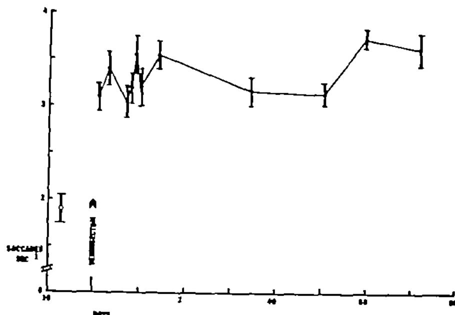


Fig 2 Polysaccadia in the vermisectomized monkey changes little with time Before surgery (I) after surgery (●) The variations in saccadic frequency after surgery are not correlated with experimental procedures

if the monkey is tested frequently Immediately after vermisectomy the time constant of nystagmus is always 6 to 10 sec and this value is not influenced by the pre surgery time constant During the first week after surgery the time constant of nystagmus increases to values in excess of 20 sec and (excepting experimentally induced temporary modifications) maintains values in this range for as long as 60 days Fig 4 shows the time course of the changes in time constant after vermisectomy

III *Modification of the vestibulo-ocular reflex by repeated testing*

After vermisectomy the vestibulo-ocular reflex is resistant to modification by repeated testing Fig 5A shows that in a normal monkey the time constant of nystagmus in the B-test decreases exponentially to about 7 sec when the monkey is repeatedly tested on one day In the first days after vermisectomy when the time constant in the B-test is low repeated testing effects a slight but sometimes statistically significant *increase* in time constant For the next 3–4 weeks after vermisectomy while the time constant in the B-test is high repeated testing either has no effect on time constant or if it does reduce the time constant cannot reduce it to a value near 7 sec Fig 4 shows the time course after vermisectomy of susceptibility to modification of the VOR by repeated testing Fig 5B shows that in the vermisectomized monkey the time constant in the B-test decreases exponentially when the monkey is repeatedly tested on one

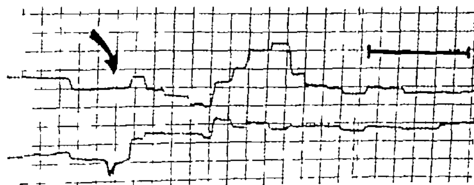


Fig 3 Polysaccadia in the vermisectomized monkey disappears when his eyes are covered At arrow the eyes are uncovered and polysaccadia returns Time bar = 1 second

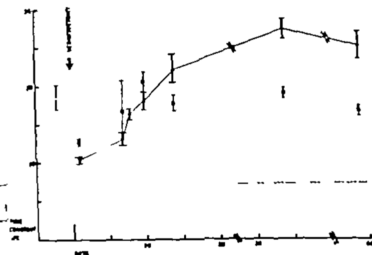


Fig. 4. The time constant of nystagmus in modified Bárány spinning test (B-test) shows regular pattern of change after ablation of the cerebellar cortex: the time constant is low during the first week after surgery then assumes high values. Before surgery (O) after surgery (●). After vermisectomy the time constant of nystagmus in the B-test is not modified in the usual way by repeated testing: immediately after surgery the time constant increase when the animal is repeatedly tested, much later the time constant decreases slightly when the animal is repeatedly tested. Time constant after repeated testing (□). Dashed line shows the 7-second nystagmus time constant to which normal monkeys may be reduced by repeated testing.

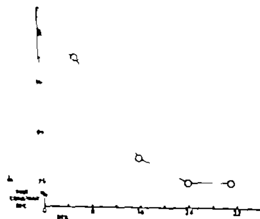
day but that it reaches a plateau at a value well above 7 seconds.

IV. *Modification of the vestibulo-ocular reflex by unidirectional optokinetic nystagmus*

After vermisectomy the VOR shows the usual modification by unidirectional optokinetic nystagmus. Fig. 6A shows for a normal monkey the asymmetry of time constant of nystagmus in the B-test induced by optokinetic nystagmus. Fig. 6B shows for a vermisectomized monkey a similar modification.

V. *Modification of the vestibulo-ocular reflex by reversing spectacles*

After vermisectomy the VOR shows the usual



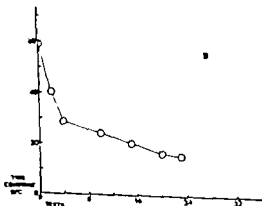


Fig. 5. Repeated B tests reduce time constant of nystagmus in the B test approximately exponentially with number of tests, both before and after vermisectomy but the modification is more complete before vermisectomy (A) than after (B). The data points shown fit an exponential curve with correlation coefficients of (A) 0.95 (B) 0.96.

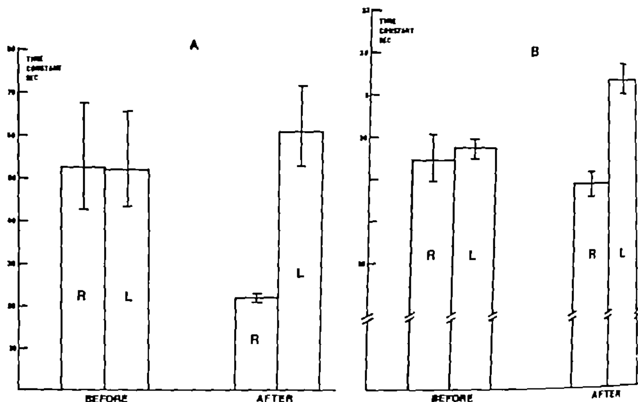


Fig 6 Optokinetic nystagmus induces asymmetry of time constant of nystagmus in the B-test both in normal and in vermisectomized monkeys. (A) In normal monkey after 15 minutes of nystagmus. (B) In vermisectomized monkey after 9 minutes of nystagmus.

modification when the monkey wears reversing prisms Our normal monkeys who wear reversing prisms show a reduction in gain or reversal of the VOR as tested by sinusoidal rotation Accompanying this is a complex modification of the pattern of nystagmus in the B-test the smooth phase direction in the B-test never reverses but the time course of nystagmus is no longer exponential and smooth phase velocity is low and highly variable These changes in the B test are shown in Fig 7 A Similar changes take place and at least as rapidly in vermisectomized monkeys who wear reversing prisms as shown in Fig 7B Our monkeys have worn reversing prisms during the first week after vermisectomy (the period when the time constant of nystagmus in the B-test is low) and during the eighth or ninth week after vermisectomy (the period when the time constant of nystagmus in the B-test is high) The modification of the VOR is similar at either time

DISCUSSION

The vermis of the cerebellum is apparently not significantly involved in gaze-holding and pursuit oculomotor functions that are severely impaired when the entire cerebellum is ablated (Westheimer & Blair 1973) or when an entire half-cerebellum is ablated (Westheimer & Blair 1974) The only oculomotor signs characteristic of the vermisectomized monkey are polysaccadia and saccadic dysmetria Ritchie (1976) has already discussed saccadic behavior after causing vermal lesions in the monkey It remains to be pointed out that the saccades in the vermisectomized monkey just as in the completely cerebellectomized monkey are normal in their dynamic characteristics even if they are not perfectly integrated into the visual-oculomotor behavior of the monkey This observation emphasizes the important distinction between those neural substrates necessary for generation of saccadic

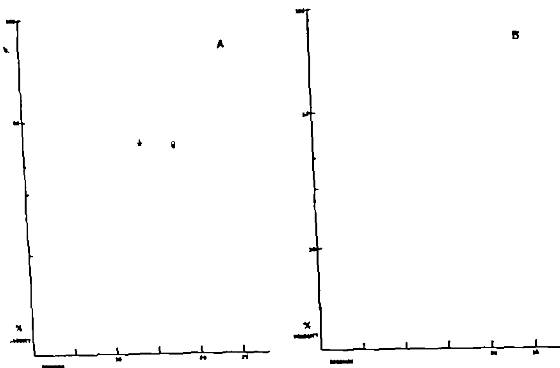


Fig. 7. Reversing prism spectacles modify the pattern of nystagmus in the B-test in normal and in vermisectomized monkeys: the time course is no longer exponential and smooth phase velocity is low and highly variable. (A) Normal monkey before (●) and after 7 days (O) and 14 days (★) wearing reversing prism spectacles. (B) Vermisectomized monkey before (●) and after 8 days (O) wearing reversing prism spectacles. Gain in vestibulo-ocular reflex, as tested by sinusoidal rotation at 0.5 Hz was, in (A) 0.6 after 7 days, and 0 after 14 days, in (B) 0.1, after 8 days. Ordinate in A and B show nystagmus velocity as % of B-test velocity step.

movements and those neural substrates that ensure that the movements generated will suit the monkey's needs.

The modified Bárány spinning test (B-test) highlights a particular feature of the vestibular ocular reflex are: the discrepancy between cupular time constant and nystagmus time constant. The time constant that governs endolymph (and thus cupular) displacement during and following unidirectional accelerations is about 6 seconds (measured from primary vestibular afferents in the squirrel monkey by Goldberg & Fernandez 1971). In *M. speciosa* the cupular time constant is probably also about 6 seconds, but in the B-test the time constant of nystagmus varies from 7 to 80 seconds and is easily modified by experimental manipulation. The difference between cupular time constant and nystagmus time constant must manifest the way in which information carried by primary vestibular afferents is processed in the central nervous system. The present experiments direct attention to the way in which vermisectomy alters this processing and alters the effect of at least one experimental manipulation which ordinarily changes this processing.

After vermisectomy the time constant of nystagmus in the B-test changes with time in a consistent pattern—it is very short (near the cupular time constant) for a few days, then becomes very long (sometimes near the maximum seen in normal monkeys) for an extended period of time. This course of events suggests that vermisectomy first inhibits, then releases from inhibition, some extra-vermal neuronal

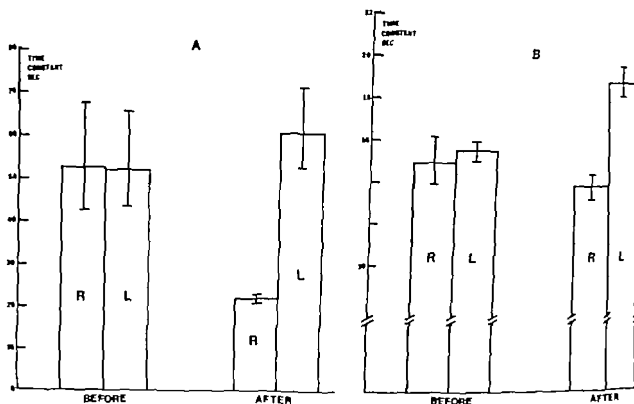
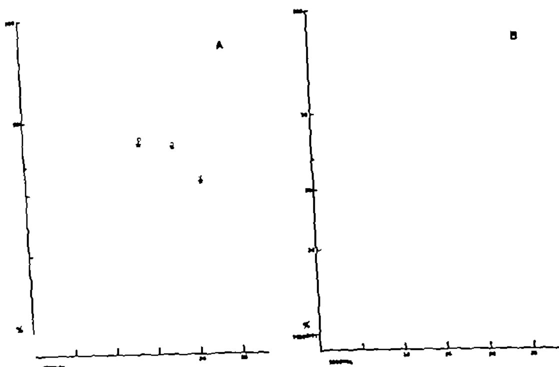


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7 Reversing prism spectacles modify the pattern of tagmus in the B-test in normal and in vermisectomized keys the time course is no longer exponential and both phase velocity is low and highly variable. (A) mal monkey before (●) and after 7 days (○) and 14 s (✱) wearing reversing prism spectacles. (B) Vermisectomized monkey before (●) and after 8 days (○) wearing reversing prism spectacles. Gain in vestibulo-ocular reflex, as tested by sinusoidal rotation at 0.5 Hz as: in (A) 0.6 after 7 days, and 0 after 14 days. In (B) 0.12 after 8 days. Ordinate in A and B shows nystagmus velocity as % of B-test velocity step

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circuitry which processes vestibular input into nystagmus velocity A parallel sequence of changes may be seen in the phenomena of spinal shock after transection of the spinal cord spinal reflexes are at first hyporeactive then hyperactive (Sherrington 1906) That the neuronal circuitry essential to generation of a long nystagmus time constant remains intact after vermisectomy is shown by the ultimate return of a long time constant and by the modifiability of the long time constant For the modifying of vestibular nystagmus visual sensory inputs (optokinetic nystagmus re versing prism spectacles) are at least as effec tive in the vermisectomized monkey as in the normal monkey In contrast repeated B-test ing does not modify vestibular nystagmus in the vermisectomized monkey to the same extent as in the normal monkey Visual inputs probably exert their effects through pathways in the flocculi (Lisberger & Fuchs 1974) and floccular pathways are spared by vermisec tomy Sensory input to the vermis is partly visual but is also proprioceptive in lobules V VI and VII and vestibular in lobules IX and X (Fuchs & Kornhuber 1969 Baker et al 1972 Gardner & Fuchs 1975 Precht Simpson & Llinas 1976) The relative failure of repeated B testing to modify vestibular nystagmus after vermisectomy may indicate that this particular modification depends on processing both of canal signals and eye movement propriocep-tive signals in the vermis of the cerebellum and that the modification results when a dis crepancy (*not accounted for by e g current* visual input) exists between canal signals and eye *movement* signals

ZUSAMMENFASSUNG

Nach Entfernung des Vermis cerebelli zeigt der vestibulo-okuläre Reflex (VOR) bei *Makaken regelmäßig* Veränderungen bei Anwendung des Báránylschen Drehtestes Ein Tag nach der Operation liegt die Zeitkonstante des Nystagmus im unteren Normalbereich steigt danach langsam an und erreicht nach einer Woche Werte im oberen Normalbereich Nachdem die Zeitkonstante sich auf dem hohen Niveau stabilisiert hat kann sie durch wiederholte Anwendung des Drehtestes nicht so stark reduziert werden, wie dies beim intakten Tier möglich ist. [...]seits bleibt die Veränderbarkeit des VOR durch Auslösung von optokinetischem Nystagmus und Anwendung von Umkehr Prismen erhalten. Diese [...]nisse lassen darauf schließen daß der Vermis [...] bei Modifikationen des VOR durch visuelle Zuflüsse [...] Rolle spielt wohl aber bei Modifikationen durch [vesti]bulare Zuflüsse

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THE MATURATION OF VESTIBULAR NYSTAGMUS *IN INFANCY AND CHILDHOOD*

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Abstract The displacements durations and velocities of the slow and fast components of both the primary and secondary nystagmus induced by constant angular acceleration were measured in 46 normal children 1 month to 11 years old There were significant changes in nystagmus parameters in respect to maturation The young infant had larger amplitude higher velocity beats than the older child during both the *primary* and the *secondary* nystagmus Parameters describing both the *primary* and the *secondary* nystagmus reached their peak values and terminated earlier in the infant than in the older child. Although the slow component velocity during the *secondary* nystagmus was much slower than during the *primary* nystagmus at all ages the secondary nystagmus/primary nystagmus ratio was significantly greater in early infancy Thus in infancy as compared with later childhood the vigor of the secondary nystagmus was disproportionately greater than the primary nystagmus These results were discussed in relation to the maturation both of vestibular responsiveness and of vestibular adaptation

This report provides quantitative data on the maturation of vestibular function during infancy and early childhood The vestibular nystagmus data are collected in a way which permits assessment of *vestibular adaptation* the magnitude of which is quantitatively indexed by the time course of the total nystagmus response including the secondary nystagmus (Young & Oman 1969 Malcolm & Jones 1970)

There is insufficient information on the time course of vestibular nystagmus in children and none on secondary nystagmus Several studies on normal children measured post rotatory nystagmus durations in 6–9-month olds (Kantner et al 1976) 3–5-year-olds (Steinberg & Rendle Short 1977) and 3–7 year-olds (Ornitz et al 1974) using the Bárány procedure which precludes stu the secondary nystagmus (Wendt 1951) sion swing and caloric stimulation have used to elicit nystagmus in infants up months old (Eviatar et al 1974) and in year-olds (Van der Laan & Oostervold l The latter study found that preadole children had lower nystagmus frequer higher amplitudes and higher velocities individuals in any older age group In the comprehensive study of maturational cha *within* the childhood years Tibbling (l *measured velocities and amplitudes* of slow and fast components of the primary tagmus following an intense brief accelera however any secondary nystagmus was reported

METHODS

Subjects

Nystagmus recordings of sufficient quality data analysis were obtained from 46 no children (24 boys and 22 girls) 1 month t years old (Table I)

Stimulation

A remotely controlled chair (Tonnies M zinische Elektronik Freiburg/Breisgau) accelerated at 10°/sec² for 18 sec const

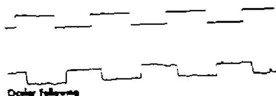
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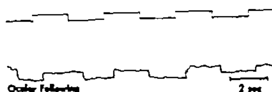
Ocular Following 10° From Center

3 YR. OLD CHILD



Ocular Following

9 MO. OLD INFANT



Ocular Following 2 sec

Fig. 2. Ocular calibration. The square wave indicates the position of the calibration light. Instantaneous motion of the light from the center of the subject' visual field 10 degrees to the right or left is indicated by downward deflection of the square wave. The 3-year-old child is following the light from center 10 degrees to the right (upward deflection of the ocular tracing). The 9-month-old infant is following from center 10 degrees to the left (downward deflection of the ocular tracing).

Fig. 1. Six-month-old infant watching calibration lights. During accelerations, head is anteroflexed by additional padding behind occiput.

velocity at 180°/sec was continued for 200 sec in absolute darkness. Proper positioning was maintained by partial fixation of the subject's head with an occipital support, an elastic head strap, and a chin rest. The chin rest was not used for infants under 14 months, who were strapped into a modified infant car seat which, in turn, was rigidly set into the rotating chair (Fig. 1). Positioning the angle of the car seat and a padded neck collar assured correct positioning of the infant's head.

Children years and older came for six sessions at 3 to 7 day intervals, and received up to six rotations (including acceleration magnitudes other than the 10°/sec² reported here) per session. Infants under 14 months came for one or two sessions and received 2 to 4 rotations per session.

Each session began with 10 min of partial dark adaptation (dark room red lights) followed by 5 min of absolute darkness before the first calibration. Ocular displacement was calibrated while the subject followed the instantaneous movement of a pinpoint red light through 10 degrees of arc to the right or left of midline visual fixation in absolute darkness. Calibration was considered adequate only if ? or more saccadic eye movements varied by less than 7% and followed the calibration light by no more than 0.6 sec (Fig. 2). Calibration was repeated within 5 min of the beginning or end of each trial. At least 8 min elapsed from the end of one rotation to the beginning of the next. An adult was in the room at all times playing with the child between rotations and singing to the child during rotations.

Singing to the child during rotations both reassured the child and counteracted drowsiness. Comparisons between trials when this procedure was used and when it was not used showed that there was no effect on ocular position or motion.

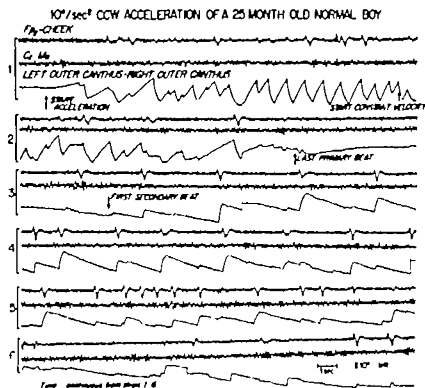


Fig 3 Example of a successful tr... ing (upper channel) EEG (midd... nel) and d c oculogram (lower ch... each panel are all consistent with ... relaxed alertness There is vigorou... ry and secondary nystagmus Tim... tinuous from panels 1 through 6 ... tion is shown in panel 1 Constant... rotation continues through the r... panels

Recording and subject monitoring

Horizontal eye movements were recorded bipolarly from silver–silver chloride electrodes at the outer canthi on a Grass 78 polygraph using a paper speed of 30 mm/sec after d c amplification Vertical eye movemen... blinks were recorded through a c am... tion from electrodes placed directly ... and below one eye EEG was recorde... one or more vertex or parieto-occipital ...

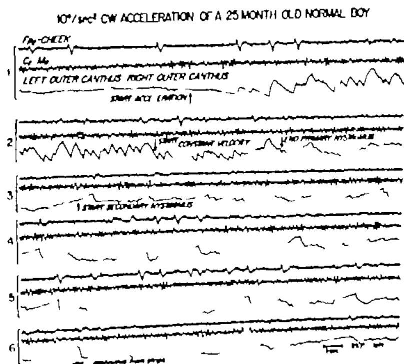


Fig 4 Example of an unsuccessf... The primary nystagmus persist f... sec after the end of accelerat on a... placed by slow irregular oscillat on... d c oculogram Blink artifacts are... in panel 3 and 6 and the EEG show... activity A the secondary nystagmu... lops it appears irregular because ... imposed low rolling eye move...

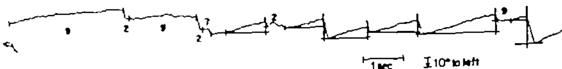


Fig 5 Example of nystagmus measurement during 14 sec of secondary nystagmus The horizontal and vertical lines associated with each nystagmus beat correspond to the duration and amplitudes of the slow and fast components. Other segments of the record are coded (9 for no ocular activity 2 for non-nystagmic eye movements and 7 for questionable nystagmus beats) until all elapsed time is accounted for

ions A closed circuit infrared TV system permitted observation of the child in absolute darkness through 180 degrees of rotation Changes from an alert to drowsy state were monitored by visual observation of facial ex pression and body posture by increases in amplitude and slow activity in the EEG by decreased blinking, and by the development of slow rolling movements in the oculogram Eye closure was monitored visually and by absence of frequent blink artifact in the vertical oculogram Head and facial movements were monitored visually and by the presence of movement artifact and muscle tension in scalp and facial leads. Trials were discarded if drowsiness, eye closure head movement or facial movement were detected by any of these procedures. Fig. 3 illustrates a successful trial while Fig 4 shows a discarded trial in which the same subject is drowsy

Dat measurement

All 18 sec of polygraph record for each trial were measured and coded by two persons independently with final judgement by consensus Four measurements were made for each beat identified as nystagmus slow component amplitude (a) slow component duration (d_s) fast component amplitude (a_f) and fast component duration (d_f) Slow and fast component velocities were calculated as $v = a_s/d$ and $v_f = a_f/d_f$ for each beat. Each 0 2 sec of the polygraph record was accounted for either by the presence of part of a measurable nystagmus beat or by a series of codes indicating the elapsed time of eye movements other than nystagmus (e g. saccades) movement artifact, questionable nystagmus (too irregular to measure) or absence of any activity in the d c oculogram (Fig 5)

Data sampling

One successful CW and CCW trial were obtained for each of the 46 subjects It was necessary to record from 84 infants and children to obtain these data. For these 46 subjects it was necessary to attempt additional recordings which were rejected due to drowsiness eye closure or excessive movement. Tables I and II indicate the rejection rate for subjects and trials relative to subject age

Data analysis

The amplitudes durations and velocities of the individual nystagmus beats were utilized in

Four subjects had more than one successful trial in each direction. For these subjects, data from two or more trials in one direction were averaged before further data analysis.

Table I *The relati e success in recording nystagmus from infants and children of different ages*

Age	Number of subjects attempted	Number of subjects successfully recorded	Percent of subjects successfully recorded
<5 months	7	5	71 4
5–7 months	13	6	46.1
8–10 months	17	6	35 2
11–13 months	18	6	33.3
2 3 years	9	9	100 0
4–6 years	18	12	66 7
9–11 years		2	100 0
Total subjects	84	46	54 7

Table II *The relative success in obtaining nystagmus recordings from the 46 infants and children who actually were recorded in relation to age*

Age	Number of trials attempted	Number of successful trials	Percent of successful trials
<5 months	15	10	66 7
5–7 months	21	12	57 1
8–10 months	31	1	38 7
11–13 months	26	12	46.2
2–3 years	49	23	46 9
4–6 years	66	36	54 6
9–11 years	8	4	50 0
Total	16	109	50.5

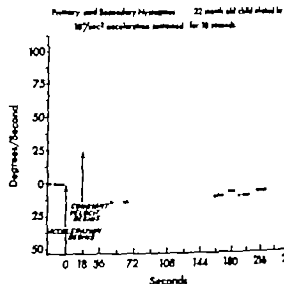


Fig 6 Slow component velocity averaged every 3
Secondary nystagmus is indicated by negative values

Table III *Nystagmus parameters calculated by summing and/or averaging nystagmus separately during each of the 3 phases of nystagmus*

	Peracceleration primary nystagmus			Postacceleration primary nystag		
	Correlation with age (or log age) r^a	Mean±1 S D 23 infants[a]	Mean±1 S D 23 children	Correlation with age (or log age) r^a	Mean±1 S D 23 infants[a]	Mean±1 S D 23 chi
Total number of beats	0.53	21 5±4.5	27 5±11 [illegible]	0 58[c]	15 0±6.4	46.
Frequency (beats per sec)	0 54	1.2±0 3	1 6±0 6[e]	0 41	1 0±0.2	1 1
Slow component						
Total displacement (deg)	−0 44	757 9±239 9	577 7±151 7[b]	0.20	368.8±172.6	448.1
Mean unit displacement (deg per beat)	−0.54	36 0±11 8	4 4±11 2[e]	−0.4[illegible]	5 8±8.8	19 8
Total duration (sec)	−0.2	11 7±1 6	11 1±1 7	0 46[b] (0.56)[c]	9 0±3 7	1 9
Mean unit duration (sec per beat)	−0.50[e]	0 6±0 1	0.5±0.2[e]	−0.29	0.6±0.1	0 6
Average velocity (deg/sec)	−0 40[b]	65 0±18 9	51 4±9 6	−0.28	40 6±12 0	31 6
Fast component						
Total displacement (deg)	−0 40[b]	757 2±251 7	567.[illegible]±158.8[b]	0 [illegible]0	363 2±175 1	445 7
Mean unit displacement (deg per beat)	−0.5[illegible]	35 9±11 9	3 8±11 1	−0 40[b]	25 1±8.3	70.5
Total duration (sec)	0.36	2 9±0.5	3 3±1 0	0 52 (0.59)[c]	1 6±0 7	6
Mean unit duration (sec per beat)	−0 16	0.1±0 03	0.1±0 05	0 03	0 1±0 03	0 1
Average velocity (deg/sec)	−0.54	63 0±80.8	18 1±58 0[e]	−0 45	31 1±66.8	175.3

The linear correlation coefficient r was calculated after log transformation of age in those instances where perus scattergrams suggested an exponential relationship between the nystagmus parameter and age
The probability that r is significantly different from zero (two-sided test) is. <0 05 <0 01 <0.001
Under 14 months old
Over 2 years old
The probability that the differences between the means of the infants and those of the children are significant two-sample t-test with separate variances and an approximate degrees of freedom solution (two-sided test) is. <0 01 <0 001

the calculation of several *parameters* during each of three *phases* of the response to acceleration The *phases* of each trial were defined as the *peracceleration primary* nystagmus the *postacceleration primary* nystagmus and the *secondary* nystagmus.

The main *parameters* which were calculated during each phase for both the slow and the fast *components* separately were (1) the total amplitude (ocular displacement) in degrees (2) the total duration (total time during which e.g. slow component displacement occurred) in seconds, and (3) the average velocity in degrees/sec The average velocity within a given phase of a single trial was calculated as

$$\frac{a_1 + a_2 + \cdots + a_n}{d_1 + d_2 + \cdots + d_n}$$

where a_i and d_i are the amplitudes and durations of the e.g. slow components of the individual nystagmus beats Additional *parameters* included the total number of beats th frequency of nystagmus in beats per secon the mean unit displacement in degrees p beat and the mean unit duration (e.g. of th slow components) in seconds per beat. Th total times taken up by absence of ocular a tivity non-nystagmic eye movements que tionable nystagmus or artifact in th nystagmogram were also calculated

Fig. 6 illustrates the time course of the ny tagmus using the slow component velociti averaged every 3 sec from a single subjec The magnitudes and the latencies from th onset of acceleration to the midpoint of the sec intervals showing the peak primary ny tagmus and the peak secondary nystagmu were calculated for each subject for tw *parameters* slow component *amplitude* p beat (peak unit displacement) and slow con ponent velocity The time course of the ny tagmus was further delineated by calculatio of the latencies from start of acceleration t the end of the primary nystagmus to the be ginning of secondary nystagmus and to th end of secondary nystagmus

The data from each subject s CW and CCV trials were averaged and these means wer *used* for comparisons based on age Becaus of the possibility of directional preponderance comparisons based on age were also made us ing the maximum values from each child's pai of CW and CCW trials Since these analyse yielded very similar results only the result based on averages of CW and CCW trials wil be reported

RESULTS

Table III shows the linear correlations be tween nystagmus parameters and age for each of the three *phases* of nystagmus Twenty o the 36 correlations were significant at the 1 % level or better Fourteen of these 20 significant correlation coefficients were negative indicat ing a general tendency for nystagmus parameters to decrease with increasing age Thus a more vigorous nystagmus in early in-

condary nystagmus		
orrelation ith age r log age[d]	Mean ± S D	
	23 infants[a]	23 children[a]
0 25	43 4±15 9	53 2±24 3
0 10	0 5±0 1	0 4±0 2
0 25	593 3 342 4	493 6±324 9
0 49	13 2±5 5	9.2±3 6[b]
0 09	35.5±17 0	45 9±21 0
0 04	0 8±0 3	1 0±0 4
0 49	16 7±7 0	9 9±2 0
(−0 55)		
0 21	652 5±359 8	593 0±348 9
0 44	14.9±5 8	11 5±3 [illegible]
0 14	3.8±1 6	5 0±2.3
0 02	0 1±0 02	0.1±0 03
−0 50	167 2±47 3	125 6±35 1

fancy is attenuated with maturation Dividing the subjects into two age groups infants under 14 months and children over 2 years generally confirmed this impression (see right hand columns of Table III)

The positive correlation coefficients in Table IV indicate that many temporal (latency) parameters increase significantly with increasing age Also the elapsed time from the end of primary to the beginning of secondary nystagmus is significantly longer with increasing age Table IV also shows that the maximum unit displacement during the primary nystagmus and the maximum velocity during the secondary nystagmus are greater in early infancy than in later childhood Fig 7 illustrates these differences In infancy the nystagmus response is more intense in respect to magnitude and timing each phase of nystagmus reaches its peak value earlier and is finished earlier Fig 8 illustrates the effect of increasing age on one nystagmus parameter the time from the beginning of acceleration to the start of secondary nystagmus increases exponentially with age with the greatest increments of change prior to 30 months

Table V shows the influence of age on the proportion of secondary nystagmus (occurring long after the stimulus of acceleration has ended) to primary nystagmus (which is more directly influenced by the acceleration) The velocity of the slow component during secondary nystagmus relative to the primary nystagmus is significantly greater during early infancy than later in childhood

In order to assess the inter relationships amongst the many nystagmus parameters and their possible clustering into subsets we performed a factor analysis on all 57 variables in Tables III IV V The data were described by four factors (1) primarily variables which involved mean unit displacement (displacement per beat) or velocity (2) parameters describing the time course of the nystagmus response (Table IV) (3) variables which involved frequency (beats per second) or mean unit duration (seconds per beat) and (4) total displacements durations or number of beats e[illegible] during the secondary nystagmus or expre[illegible] as ratios of the secondary to the primary [illegible]tagmus A stepwise discriminant analysis [illegible] also performed on all variables using th[illegible] infants and 23 children as groups. The [illegible] variables (latency to the end of secondary [illegible]tagmus and the peak (maximum) slow c[illegible]ponent velocity during the secondary ny[illegible]mus) which together correctly classified [illegible] of the subjects into their age groups came [illegible] the first two factors identified by the fa[illegible] analysis

Directional preponderance

The possibility that the occurrence of d[illegible]tional preponderance might vary with age [illegible] tested by computing the value

$$\left| \frac{CW-CCW}{CW+CCW} \right|$$

for each of the nystagmus parameters list[illegible] Tables III and IV Only eight parame[illegible] showed significant differences between th[illegible] fants and the children and seven of these [illegible]ferences were marginal ($p<0.05$) [1] Howe[illegible] in all but one of these parameters the c[illegible]putation was greater for the infants than [illegible] older children Since in most subjects t[illegible] was only one CW and one CCW trial t[illegible] relatively few differences may represent e[illegible] a greater tendency toward directional pre[illegible]derance or a greater trial to trial variabili[illegible] greater lability of response) in younger [illegible]jects

Habituation

Since the children over 2 years old rece[illegible] more rotations than the infants under [illegible] months the possible influence of habitua[illegible] in the older children was studied For [illegible]

---

[1] Slow and fast component average velocity and [illegible] component total duration, mean unit and total dis[illegible]ment during the postacceleration primary nystagmus [illegible] fast component average velocity mean unit displace[illegible] and total duration during the secondary nystagmus.

Only the fast component mean unit displacement d[illegible] the postacceleration primary nystagmus showed a significant difference between the two age g[illegible] ($p<0.002$)

able IV *Nystagmus parameters representing the time course of the response from the begin-'ng of acceleration to the end of secondary nystagmus*

	orrelation ith log age (or age)[a] r	Mean±1 S.D 23 infants[d]	23 children
eak (maximum) value of slow component unit displacement, *primary* nystagmus			
Displacement (deg per beat)	−0.35 (−0.43)[e]	50 6±17 1	39 0±15.6[e]
Latency from beginning acceleration (sec)	0.47[b]	12.0±4.0	16.7± 6.3[e]
Peak (maximum) value of slow component velocity *primary* nystagmus			
Velocity (deg/sec)	−0.20	90.9±32.7	76.6±16.0
Latency from beginning acceleration (sec)	0.43	14 0±2.0	15 7±1 7[e]
Latency beginning acceleration to end of *primary* nystagmus (sec)	0 58[c]	34 4±5.8	40 0±6.6[e]
Elapsed time from end of *primary* to beginning of *secondary* nystagmus (sec)	0.53[c] (0.46)[e]	3.9±3.5	8.5±4.5[e]
atency beginning acceleration to start f secondary nystagmus (sec)	0 75[c]	38.2±5.9	48 5±7 4[f]
ak (maximum) value of slow component unit isplacement, *secondary* nystagmus			
Displacement (deg per beat)	−0.32[a] (−0 41)[e]	33.2±14.8	77.–±10.7
Latency from beginning acceleration (sec)	0 56[c]	67 9_12.1	92.1±18.7[f]
ak (maximum) value of slow component velocity *secondary* nystagmus			
Velocity (deg/sec)	−0 48[b]	38.3±21 1	19.2±6.0[e]
atency from beginning acceleration (sec)	−0.49[c]	68.1±16.9	98.1±32.4
atency from beginning acceleration to end of *secondary* nystagmus (sec)	0.71	125 1±23.3	174.1±23 2[f]

The linear correlation coefficient was calculated after log transformation of age except in those instances where ersual of the scattergrams suggested linear relationship between the nystagmus parameter and age. The majority of nystagmus parameters in this table showed an exponential relationship to age
The probability that is significantly different from zero (two-sided test) is: <0 05 <0 01 <0 001
Under 14 months old
Over years old
The probability that the differences between the means of the infants and those of the children are significant, using two-sample -test with separate variances and an approximate degree of freedom solution (two-sided test) is:
<0 05 <0 01 <0 001

analysis the maximum value for each of six representative nystagmus parameters was identified for each of the 21 children between 23 and 83 months of age These 21 trials were then dichotomized into those occurring during the first session in the laboratory versus later days and those occurring relatively early in the session (first to third trial) versus later in the session (fourth trial or later) A two-by two analysis of variance (with age as a continuous covariable) revealed no significant dependence on either order of session or trial within session for any of the variables

DISCUSSION

There are three major findings of this investigation First, the young infant has larger amplitude higher velocity beats than the older child during both the *primary* and *secondary* nystagmus evoked by constant angular acceleration Second parameters describing both the *primary* and *secondary* nystagmus reach

Slow component total displacement, mean unit displacement, and average velocity each during the perac celeration primary and during the secondary nystagmus.

fancy is attenuated with maturation Dividing the subjects into two age groups infants under 14 months and children over 2 years generally confirmed this impression (see right hand columns of Table III)

The positive correlation coefficients in Table IV indicate that many temporal (latency) parameters increase significantly with increasing age Also the elapsed time from the end of primary to the beginning of secondary nystagmus is significantly longer with increasing age Table IV also shows that the maximum unit displacement during the primary nystagmus and the maximum velocity during the secondary nystagmus are greater in early infancy than in later childhood Fig 7 illustrates these differences in infancy the nystagmus response is more intense in respect to magnitude and timing each phase of nystagmus reaches its peak value earlier and is finished earlier Fig 8 illustrates the effect of increasing age on one nystagmus parameter the time from the beginning of acceleration to the start of secondary nystagmus increases exponentially with age with the greatest increments of change prior to 30 months

Table V shows the influence of age on the proportion of secondary nystagmus (occurring long after the stimulus of acceleration has ended) to primary nystagmus (which is more directly influenced by the acceleration) The velocity of the slow component during secondary nystagmus relative to the primary nystagmus is significantly greater during early infancy than later in childhood

In order to assess the inter relationships amongst the many nystagmus parameters and their possible clustering into subsets we performed a factor analysis on all 57 variables in Tables III IV V The data were described by four factors (1) primarily variables which involved mean unit displacement (displacement per beat) or velocity (2) parameters describing the time course of the nystagmus response (Table IV) (3) variables which involved frequency (beats per second) or mean unit duration (seconds per beat) and (4) total displacements durations or number of beats either during the secondary nystagmus or expressed as ratios of the secondary to the primary nystagmus A stepwise discriminant analysis was also performed on all variables using the [illegible] infants and 23 children as groups The two variables (latency to the end of secondary nystagmus and the peak (maximum) slow component velocity during the secondary nystagmus) which together correctly classified 89[illegible] of the subjects into their age groups came from the first two factors identified by the factor analysis

Directional preponderance

The possibility that the occurrence of directional preponderance might vary with age was tested by computing the value

$$\left| \frac{CW - CCW}{CW + CCW} \right|$$

for each of the nystagmus parameters listed in Tables III and IV Only eight parameters showed significant differences between the infants and the children and seven of these differences were marginal ($p<0.05$) [a] However in all but one of these parameters the computation was greater for the infants than the older children Since in most subjects there was only one CW and one CCW trial these relatively few differences may represent either a greater tendency toward directional preponderance or a greater trial to trial variability (greater lability of response) in younger subjects

Habituation

Since the children over 2 years old received more rotations than the infants under 14 months the possible influence of habituation in the older children was studied For this

[a] Slow and fast component average velocity and fast component total duration mean unit and total displacement during the postacceleration primary nystagmus, and fast component average velocity mean unit displacement and total duration during the secondary nystagmus

Only the fast component mean unit displacement during the postacceleration primary nystagmus showed a strongly significant difference between the two age groups ($p<0.002$)

Table V *The proportion of secondary to primary nystagmus (the ratio secondary nystagmus/primary nystagmus)*

	Correlation with age (or $\log_{10}$ age)[1] r[2]	Mean ± 1 S.D. 23 infants[3]	23 children[4]
Total number of beats	−0.35*	1.25±0.43	1 12±0.46
Slow component			
Total displacement	−0.16	0.52±0.20	0.52±0.27
Peak (maximum) unit displacement	0.17	0.65±0 18	0.86±0.39*
Total duration	−0 05	1 72±0.63	2.05±0 89
Average velocity	−0.37* (−0.51)*	0.31±0.07	0.24±0 05*
Peak (maximum velocity)	−0.55 (−0.65)*	0.42±0.12	0.25±0.07*
Fast component			
Total displacement	−0.11	0.59±0 22	0.62±0.28
Total duration	−0 24	0.88±0.31	0.87±0.34
Average velocity	0.20	0.67±0.11	0.72±0.14

[1] The linear correlation coefficient was calculated after log transformation of age in those cases where perusal of the scattergrams suggested an exponential relationship between the nystagmus parameter and age.
[2] The probability that r is significantly different from zero (two-sided test) is: <0.05 <0.01 <0.001
[3] Under 14 months old.
[4] Over 2 years old
[5] The probability that the differences between the means of the infants and those of the children are significant, using two-sample t-test with separate variances and an approximate degrees of freedom solution (two-sided test) is: <0.05 <0.01 <0.001

When, during development, do vestibular responsiveness and vestibular adaptation appear and mature? The vestibular system is both anatomically complete and functionally responsive at or before birth. The endolymphatic and bony labyrinths (Dayal et al. 1973) and the number of myelinated vestibular nerve fibers (Bergstrom 1973) and vestibular hair cells (Rosenhall 1972) are mature at birth. Newborn infants respond to acceleration: the eyes deviate in the expected direction of the slow component (Tibbling, 1969) although the fast component, with its probable pontine origin (Cohen, 1972) may not occur.

Groen (1963 1965) studied postrotatory nystagmus durations and thresholds in two infants using Frenzel glasses. His impression of complete development of inhibition by 3 months of age may have been due to the suppressive effect of light, even in the absence of fixation (Ornitz et al. 1974 Levy et al. 1977) rather than to maturation of a central regulatory mechanism. The data in the present report and those of Tibbling (1969) both collected in absolute darkness demonstrate a continuing decrease of mean unit displacement and velocity of both the slow and fast components of the primary nystagmus up to at least 7 years of age suggesting the continuous development of a modifying influence throughout early childhood. In addition our data show similar decrements of the *secondary* nystagmus.

It should be noted however that measures of the time course of the nystagmus response (latencies and durations) and also the slow component velocity of the secondary nystagmus tend to changes exponentially with age with the greatest changes prior to 30 months (see e.g., Fig. 8) Thus there may be two maturational sequences, one involving primarily the nystagmus displacement, and the mean velocity of the *primary* nystagmus develops gradually (linearly with respect to age) throughout early childhood the other involving timing parameters, and also the slow component velocity of the *secondary* nystagmus tends to mature more rapidly during the first 30 months of life. We suggest the possibility that the first sequence involves the maturation of vestibular responsiveness while the second

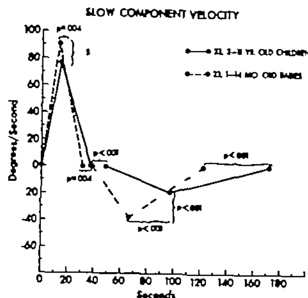


Fig 7 Comparison of slow component velocity in children and babies. The graphed values are group averages. Negative values indicate the secondary nystagmus. The probabilities (p) indicate the significance of the differences between the magnitudes and latencies in the two age groups.

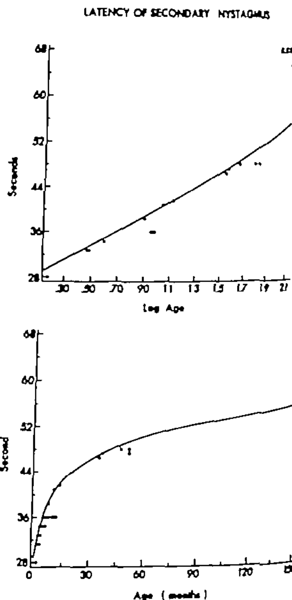


Fig 8 Latency to onset of secondary nystagmus plotted both as a function of log age and as a function of age (same data in both graphs)

their peak values and terminate earlier in the infant than in the older child Finally although the slow component velocity during the *secondary* nystagmus is much slower than during the *primary nystagmus at all ages* the secondary/primary nystagmus ratio is significantly greater in early infancy This means that in infancy as compared with later childhood the vigor of the secondary *nystagmus is disproportionately* greater than the primary nystagmus These results will be discussed in relation to the maturation both of vestibular *responsiveness* and of vestibular adaptation

For the purposes of this discussion vestibular *responsiveness* will refer to the primary response of the vestibular system to stimulation *This is the response which is characterized* by the simple second order torsion pendulum model (Van Egmond et al 1949) and is indexed by the slow component velocity of the primary nystagmus in response to brief accelerations Vestibular *adaptation* (the regulation or limitation of vestibular responsiveness) refers to the short term (several to many seconds) changes in response to a continuing stimulus The peaking of the slow component unit displacement and velocity prior to termination of acceleration particularly in the infants (Table IV) suggests that in the imma*ture human at least* vestibular adaptation to a continuing stimulus is occurring during the primary nystagmus Adequate characterization of this adaptation requires supplementation of the torsion pendulum equation with an *appropriate adaptation term* (Young & Oman 1969 Malcolm & Jones 1970) This adaptation term also accounts for the secondary nystagmus which can therefore be used to index this aspect of the vestibular response

secondary nystagmus relative to primary nystagmus implies greater vestibular adaptation. Our results therefore suggest that adaptation is greatest in early infancy and that maturation involves a progressive reduction of this neurophysiologic function during the childhood years. What is a possible function of more vigorous vestibular adaptation during early infancy? We might speculate that strong adaptation is protective when the infant is preambulatory and subject to passive motion and therefore no unpredictable stimulation of its vestibular apparatus.

Perusal of Table III reveals that during the secondary nystagmus, the fast component total displacement is considerably greater than the slow component total displacement. This result is consistent with the work of Honrubia et al. (1977) who found that the average position of the eye is biased toward the side of the orbit determined by the direction of the fast component. For the children the eyes appear to have moved on the average 118 degrees further in the direction of the *fast than the slow* component. Individual children may have considerably larger displacements. Since this is anatomically impossible, we examined all recorded eye movements for one subject. Fig. 9 plots, for this subject, all ocular displacements. It can be seen that those segments of the record which we had coded as apparent absence of ocular activity actually represent slow deviations in the direction of the slow components of nystagmus—deviations which, when summed with those of the slow components, nullify the apparent discrepancy between the fast and slow component displacement (Fig. 9 inset). These very slow deviations were not followed by a fast component, thus they did not fit our definition of nystagmus, i.e., a slow deviation followed by a fast deviation in the opposite direction. In a subsequent report (in preparation) we will provide more extensive data on this finding and its possible relation to maturation.

Malcolm & Jones (1970) find no discontinuity (no period without nystagmus) between the end of primary nystagmus and the beginning of secondary nystagmus in their adult data. They treat this finding as evidence against the existence of a threshold for cupula deviation. Our data, however, show (Table IV) such a discontinuity* and the elapsed time free of nystagmus increases significantly with increasing age. Further research will be required to determine whether magnitude of acceleration or other factors can account for these differences.

ACKNOWLEDGEMENTS

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The statistical consultation of Dr Donald Guthrie is gratefully acknowledged. Skilled technical assistance was provided by Mrs Amy Mo, Miss Laurie Muscatel, and Mrs Susan Olson.

ZUSAMMENFASSUNG

Die Amplituden, Dauer und Geschwindigkeiten der langsamen und schnellen Komponenten des durch eine konstante Winkelbeschleunigung induzierten primären und sekundären Nystagmus wurden in 46 Kindern im Alter von 1 Monat bis 11 Jahre gemessen. Die Befunde wiesen bedeutende Unterschiede der Nystagmusparameter im Verhältnis zum Reifegrad der Kinder auf. Das Kleinkind zeigte größere Amplituden und Geschwindigkeiten während des primären und auch des sekundären Nystagmus als das ältere Kind. Die Parameter des primären sowie auch des sekundären Nystagmus erreichten ihren höchsten Wert früher beim Kleinkind als bei älteren Kindern und erlöschten auch früher. Obwohl die Geschwindigkeit der langsamen Komponente während des sekundären Nystagmus bei allen Altersgruppen viel langsamer war als während des primären Nystagmus, war das Verhältnis sekundärer/primärer Nystagmusgeschwindigkeit bedeutend größer im Säuglingsalter. Daher war die Stärke des primären Nystagmus unverhältnismäßig größer als die des sekundären Nystagmus, beim Kleinkind im Vergleich zu späterem Kindesalter. Die Befunde sind in bezug auf die Entwicklung der vestibulären Erregbarkeit sowie der vestibulären Anpassung besprochen.

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*A fact possibly explained by the finding of Law et al. (1977) that the incidence of the fast component depends, in part, on the velocity of the slow component.

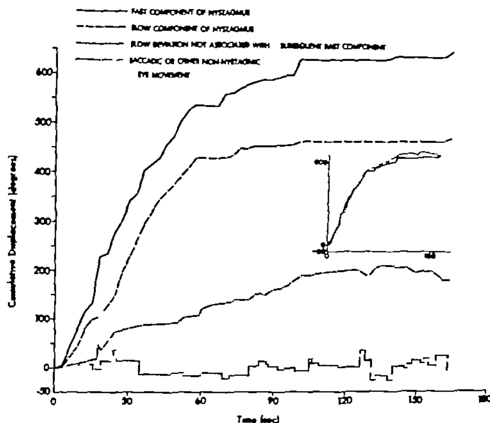


Fig 9 Cumulative ocular displacement during secondary nystagmus from a single trial from one subject The inset compares the fast component displacements (—) with the sum ( ) of the slow component displacements and the displacements due to slow deviations not associated with subsequent fast components

sequence involves the more rapid maturation of vestibular adaptation

Fig 7 illustrates the influence of maturation on the slow component velocity of the secondary nystagmus The immature response is not only more vigorous but begins peaks and terminates earlier with increasing maturation there is a temporal extension coupled with a decreased vigor of the response

Although the genesis of the secondary nystagmus is not completely understood it clearly occurs when there is no longer an *external* stimulus to the vestibular end-organ Therefore the secondary nystagmus must be generated by an *internal* stimulus or by a memory of the preceding stimulation Since there is in the adult a quantitative relationship between slow component velocities during the secondary and the primary nystagmus (Malcolm & Jones 1970) our finding that the secondary nystagmus/primary nystagmus ratio decreases with age suggests an excessive (or inadequately inhibited) *internal* stimulus in the younger infants

The *secondary* nystagmus has been attributed to an adaptive process which is superimposed on the primary vestibular response to acceleration (Young & Oman 1969 Malcolm & Jones 1970) Malcolm & Jones (1970) have described this adaptive process in terms of a shifting reference level which constantly changes so as to minimize the difference between the distortion of the cupula induced by the acceleration and the reference level itself The time course of the nystagmus (measured as change in slow component velocity) and the appearance of the secondary nystagmus in response to a prolonged acceleration of sufficient magnitude can be thought of as determined by the modification of the response of the cupula by this adaptive influence The shape of the curve of the hypothesized reference level (the adaptive influence) with respect to time (Malcolm & Jones 1970) is such that a greater magnitude of the reference level results in a smaller primary nystagmus and a larger secondary nystagmus Thus greater slow component velocity during

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PERENNIAL ATOPIC RHINITIS AS AN EARLY STAGE OF BRONCHIAL ASTHMA

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Abstract Etiologic factors and incidence of bronchial hyperreactivity as stigma of bronchial asthma were studied in 237 patients suffering from perennial atopic rhinitis. All patients underwent detailed laryngologic and allergologic examination and pulmonary function tests at rest, after exercise and after histamine inhalation. Most often the patients were sensitized to house dust, animal epidermis and fur environmental dusts, poultry dander or fungal allergens. Bronchial hyperreactivity typical of bronchial asthma, was observed in 48.52% of patients. Results were analysed statistically. It was concluded that early institution of causal therapy can cure the symptoms of rhinitis and prevent evolution of the disease into atopic bronchial asthma in patients already suffering from bronchial hyperreactivity

Perennial atopic rhinitis and allergic bronchial asthma, regarded as two different disease entities are of interest to various medical specialities e.g. otolaryngology and internal medicine According to a number of authors the two diseases coexist in 17 49% to 85 30% of cases in different populations (Blair 1974 Bystrzanowska et al 1976 Dawson et al. 1969 Koivikko 1974 Mart nowski & Gniazdowski 1976 Özkaragöz, 967 Pearson, 1973 Romański & Jonak 1969 Strömme 1966)

The majority of authors believe that symptoms of nasal allergy precede bronchial asthma (Gay 1946 Hansen, 1957 Kogan 1951 Sielużycki 1963) In Trofimenko s material (1975) symptoms of nasal allergy preceded bronchial asthma in 54 4% of cases appeared simultaneously with bronchial asthma in 36.9% whereas bronchial asthma appeared first in only 8.7% of cases In Sielużycki's material the respective proportions were 64 18% 22 39% and 13 43% and in our own patients 80.56% 15.28% and 4 18% The mean duration of the interval between appearance of perennial atopic rhinitis and onset of bronchial asthma in our patients was about 12 years

According to Herxheimer (1975) bronchial asthma in the course of perennial atopic rhinitis is so frequent that every case of rhinitis should be regarded as a serious warning of the possibility of bronchial asthma. Other authors regard rhinitis as a preasthmatic state (Romański 1976 Trofimenko 1975) Halpern (1968) and Romański (1976) believe that perennial atopic rhinitis and atopic bronchial asthma are actually symptoms of the same disease developing at two different levels of the respiratory system

This raises the questions whether clinical coincidence of these two diseases has a common cause and whether predisposition to bronchial asthma can be predicted in patients suffering from perennial atopic rhinitis

The tendency to react with bronchospasm to various environmental stimuli is a well-known symptom common to all bronchial asthma patients Romański (1976) regards this symptom as a stigma of asthmatic disease Although the pathogenesis of bronchial asthma is not the same in all cases of this disease

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Table I *Tests with mixtures of inhalatory allergens*

No of allergen mixture	Name of allergen mixture	Number of allergens in mixture (=84)	Patients with strongly positive skin reactions (+++–++++)					
			Women	% women	Men	% men	Total	% of examined patients (N=237)
1	Common household dust	1	96	66.67	59	63.44	155	65.40
2	Feather from feather beds	1	38	26.39	15	16.13	53	22.36
3	Animal dander	7	40	27.78	18	19.35	58	24.47
4	Dander from domestic herds	3	13	9.03	9	9.68	22	9.28
5	Trees 1	1					–	
6	Trees	9	7	4.86	2	2.15	9	3.80
7	Trees 3	6	3	..08			3	1.27
8	Shrubs	3	1	0.69	4	4.30	5	2.11
9/10	Grasses	16	12	8.33	11	11.83	23	9.70
11	Weeds	7	9	6.25	4	4.30	13	5.48
12	Environmental dust 1	3	17	11.81	11	11.83	28	11.81
13	Environmental dust II	8	5	3.47	4	4.30	7	2.95
14	Moulds 1	8	6	4.17		2.15	8	3.37
15	Moulds 2	7	6	4.17	3	3.23	9	3.80
16	Moulds 3	4	5	3.47	5	5.38	10	4.22

stages of the disease have been described. paroxysmal catarrhal vasodilative chronic edema, hyperplastic and polypomatous (Gnlazdowski 1977 Lichaczew & Goldman, 1967)

Mathematical analysis of the data showed significant correlation between age and duration of illness of the subjects and clinical stage of the disease

Of 38 patients (16.04%) who were under treatment for recurrent purulent sinusitis, 15 (39.47%) were operated on

2 *Allergologic Studies*

Test results with a panel of inhalatory allergens are set out in Table I.

The allergens encountered most often were house dust (pos. 1) animal dander (pos 3) and feathers (pos 2) Sensitization to environmental dust (pos 12) poultry dander (pos. 4) and fungal allergens (pos 14–16) was not uncommon Hypersensitivity to seasonally occurring plant allergens (pos 5–11) was associated in 33 cases (13.92%) with hypersensitivity to substances constantly present in the environment.

In all cases natural or provoked nasal test confirmed true clinical hypersensitivity to the various inhalatory allergens

Serum levels of IgE were elevated in 189 subjects (81.47%) Using the PRIST method, IgE levels between 100 and 500 units/ml of serum were demonstrated in 51 cases, between 501 and 1000 units/ml in 31 and over 1000 units/ml in 97 cases

More or less numerous eosinophils were observed in nasal smears in all cases, depending on whether the material was obtained during or after an attack of perennial atopic rhinitis Eosinophils were numerous in the cytologic picture of secretion at the culmination of or soon after an acute allergic attack of perennial atopic rhinitis in all cases, but present in variable numbers in material from the period between attacks Providing multiple smears were examined eosinophilia was observed in all cases In the peripheral blood, eosinophilia was present in 53.16% of our patients

characteristic experimentally reproducible bronchial hyperreactivity to histamine (Kreukniet & Pijper 1973) and cholinergic substances (Chai et al 1975 Spector & Farr 1975) can be observed in all cases and in a majority of cases also to physical exercise (Anderson et al 1975 Dziedziczko 1975)

On the basis of these data, the present study was undertaken on the causal factors of perennial atopic rhinitis and incidence of bronchial hyperreactivity as a stigma of bronchial asthma in our patients

MATERIAL

From the population of patients being treated for perennial atopic rhinitis at the Otolaryngological Section of the Outpatient Department of Allergic Diseases in Bydgoszcz in the years 1973 to 1977 a group of 237 patients age 15 to 54 years (144 women 60 76% $\bar{x}$ = 32 98 years 93 men 39 24% $\bar{x}$ = 27 40 years) was selected All patients had indisputable symptoms of perennial atopic rhinitis and no internistically detectable disorders of the lower respiratory tract or systemic disease with a possible influence on respiratory function In the examined patients were 45 (18 98%) pupils and students 75 (31 65%) intellectual workers 108 (45 57%) manual workers and 9 (3 80%) farmers A history of contact at work with physical or chemical irritants of the respiratory tract was elicited in 66 patients (27 85%)

The control group for the spirographic studies in perennial atopic rhinitis consisted of patients examined at our Clinic (Dziedziczko 1976) suffering from atopic bronchial asthma (106 patients) or entirely healthy (128 subjects)

METHODS

Clinical study in all cases included

1) Subjective objective otolaryngologic and internistic roentgenologic and laboratory studies

2) Allergologic studies (*a*) intradermal skin tests with allergens produced by SEVAC Co (Czechoslovakia) (*b*) provocation tests; (c) serum IgE level by the RIST method in H, and by the PRIST method in 218 patients (*d*) eosinophilia in nasal smears and in peripheral blood

3) Spirometric studies at rest. vital capacity (VC) one second forced expiratory volume (FEV_1) expiratory total time (ETT) spirographic index (SI) of Dubois de Montreynaud (Medicor expirograph) and after exercise of PWC_{170} type (Monark bicycle ergometer) and inhalation of histamine in rising doses (ultrasonic nebulizer TUR USI 31) by the method described by Dziedziczko (1976) The spirographic parameters were calculated as percentage decrease A decrease of the value of FEV_1 by more than 10% was considered significant. Bronchial hyperreactivity was scored as follows extremely high (+++) very high (++) and high (+)

Results were analyzed statistically using the chi square test (Gniazdowski 1977)

RESULTS

1 Physical Examination

In the group of patients with perennial atopic rhinitis the course of the disease was mild in 74 (31 22%) moderately severe in 1[illegible] (46 42%) and severe in 53 (22 36%) patients A family history of allergic disease was elicited from 163 patients (66 78%) including 1[illegible] (43 04%) in whom several family members suffered from allergic disease Allergic disease of other organs or systems preceding or coincident with symptoms or perennial atopic rhinitis was observed in 100 patients (4[illegible]) urticaria in 74 (31 22%) Quincke s edema 25 (10 55%) drug allergy in 14 (5 91%) alimentary allergy in 9 (3 80%)

Besides local symptoms of perennial atopic rhinitis numerous general symptoms are known Clinical course of the illness state of the nasal mucous membrane and roentgenographic picture of the paranasal sinuses conform to a pattern on the basis of which s

...s	% of examined patients (N=237)
	60.76
...	39.24
...0	100.0
...	

family history of allergy in only one member and 5 in several members (Table V)

No patient with a mild course of perennial atopic rhinitis showed an increase in bronchial hyperreactivity after exercise Of patients with moderately severe course 8 had positive exercise test results and of those with a severe course 4 patients (Table VI)

The results of the exercise tests in various stages of perennial atopic rhinitis were positively correlated with the stage of advancement of the disease Five of 12 patients in the polypomatous stage had positive exercise tests.

Results of exercise tests in different professional groups showed bronchial hyperreactivity in 1 farm worker 7 intellectual workers 2 pupils and students and 2 manual workers

Of 66 patients (27 85%) exposed to irritant substances at their place of work 3 (4.55%) had positive exercise test results showing bronchial hyperreactivity

(b) *Inhalatory histamine provocation tests*

Inhalation of histamine in a dose of 0.125 mg/ml for 3 min induced bronchospasm in 57 patients (24 05%) (very high bronchial hyperreactivity ++) and after inhalation of 0.25 mg/ml in 46 patients (19 41%) (high bronchial hyperreactivity +) (Table II) Bronchospasm after histamine was observed most often in patients over 30 years of age (Table III) and more often in women than in men

High (+) and very high (++) bronchial hyperreactivity were most frequent in patients in whom the first clinical symptoms of allergic rhinitis appeared between the ages of 10 and 19 years (Table IV)

Fourteen (18 9?%) of 74 (31.22%) patients without a history of hereditary allergy showed high (+) and very high (++) bronchial hyper reactivity including 8 women (5.5%) and 6 men (6.45%) Of 163 patients (68.78%) with family histories of hereditary allergy 89 (54 60%) showed bronchial hyperreactivity 62 women (43 05%) and 27 men (29 03%) (Table V)

In our population high (+) and very high (++) hyperreactivity were most frequent in patients with moderately severe clinical course of illness (Table VI)

The results of histamine provocation in different stages of perennial atopic rhinitis in patients with very high (++) bronchial hyper reactivity showed significant positive correlation between the stage of advancement of the disease and numbers of patients in the groups (in the hyperplastic stage 50 88%) In patients with high (+) bronchial reactivity correlation of this degree was not observed.

The test of provocation by inhalation of histamine was positive in 22 (48 89%) of pupils and students 26 (34 66%) intellectual workers 52 (48 15%) manual workers and 3 (33.33%) farmers

In 66 patients exposed to respiratory irritant substances during work, positive histamine tests were obtained in 31 (46 97%) including 21 women (31.82%) and 10 men (15 15%)

(c) *Spirographic results in control groups*

The Laboratory of Pathophysiology of the Respiratory and Circulatory Systems Internal and Allergic Diseases Clinic Medical Academy in Gdańsk (Bydgoszcz Branch) performed the

Table II *Bronchial reactivity in the examined patients*

No	Sex	Number of patients: without bronchial hyper reactivity ($n=122$)		with bronchial hyperreactivity TH (total hyperreactivity) = 155						
		(−)	% n	+ $n=46$	% n	++ $n=57$	% n	+++ $n=12$	% n	Total (TH 115)
1	Females	66	54 10	31	67.39	40	70 18	7	58.33	78
	% women	45 83		21.53		27 78		4.86		54 17
2	Males	56	45 90	15	32.61	17	29 82	5	41 67	37
	% men	60 21		16 13		18.28		5.38		39 79
Total		122	100 0	46	100 0	57	100 0	1?	100.0	115
% of examined patients ($N=237$)		51 48		19 41		24 05		5 06		48 52

3 *Spirographic Studies*

Spirographic studies showed bronchospasm typical of bronchial asthma in 115 patients i e 48 52% of the study population

(a) *Exercise tests*

Extreme (+++) bronchial hyperreactivity was observed in 12 cases (5 06%) namely in 7 women (4 86%) and 5 men (5 38%) (Table II) Only one of the 12 patients was in the age group under 30 years and 4 in the g under 40 years (Table III) Clinical sympt of perennial atopic rhinitis in 6 patients of group appeared before the age of 15 in 5 tients between the ages of 20 and 30 an only one above 45 years (Table IV)

In the group of patients without a hist of hereditary allergic diseases a positive fi ing in the exercise tests was obtained in c man Of the remaining 11 patients 6 ha

Table III *Age of the examined patients and bronchial reactivity*

No	Age group	Number of patients: without bronchial hyper-reactivity ($n=122$)		with bronchial hyperreactivity TH (total hyperreactivity) = 155									% of examine patients ($N=237$)
		(−)	% n	+ $n=46$	% n	++ $n=57$	% n	+++ $n=12$	% n	Total (TH=115)	% TH	All patients	
1	15–19	38	31 14	3	6.52	2	3.51	–	–	5	4.35	43	18.14
2	20–24	28	22 95	4	8 70	5	8 77	–	–	9	7 83	37	15.61
3	25–29	28	22 95	10	21 74	5	8 77	1	8.33	16	13.91	44	18 56
4	30–34	1	9 84	7	15.22	7	12.28	1	8.33	15	13 04	27	11.39
5	35–39	5	4 10	8	17.39	9	15 79	2	16.67	19	16.52	24	10 13
5	40–44	4	3 28	6	13 04	12	1 05		16 67	20	17 40	24	10 13
6	45–49	3	.46	5	10 87	9	15 79	3	25 00	17	14 78	20	8 44
8	50–54	4	3 28	3	6 5?	8	15 04	3	.5 00	14	12.17	18	7 60
Total		122	100 0	46	100 0	57	100 0	12	100.0	115	100 0	237	100 0
% of examined patients ($N=237$)		51 48		19 41		24 05		5 06		48.52		100 0	

pa- ts	% of examined patients (N –37)
	5 06
	6 75
	13 50
	18 99
	15 6
	12 66
	9 70
	9 28
	5 91
	1 6
	0.84
	100 0
) 0	

DISCUSSION

1 With the passing years the clinical picture of perennial atopic rhinitis is changing. As a result of evolution of the disease the mucous membrane of the nose and paranasal sinuses which at first was only swollen (vasodilatation) and edematous becomes hypertrophic and develops irreversible anatomic proliferative and polypomatous changes. This situation is probably due to changing pathomechanism of perennial atopic rhinitis in the course of evolution of the disease The symptoms of disease at first are caused exclusively by an allergic reaction of the immediate type in response to contact with exogenous allergens but may become more acute under the influence of non-immunologic complications (e g. disorders of innervation of blood vessels or development of a different type of allergy e g bacterial) (Bram et al. 1974 Gniazdowski 1978 Taylor 1973 Taylor & Shivalkar 1971) These complex reactions lead to chronic hyperplastic–hypertrophic (suppurative poly pous) rhinopansinusitis (Hansel 1977) and may be the main cause of evolution in the course of perennial atopic rhinitis and the differences between the clinical stages of the disease described above

We believe that in the evaluation of perennial atopic rhinitis a thorough examination of the state of the paranasal sinuses is of paramount importance and that the application of strict criteria of advancement of the disease can give a better understanding of the phenomena observed and can be helpful in planning therapy

2 In the clinical material of the present study it is evident that, as a rule perennial atopic rhinitis developed in predisposed individuals with a hereditary predisposition to atopy high levels of total IgE in the blood serum, symptoms of coexisting allergy in other organs and eosinophilia in nasal smears and peripheral blood Symptoms of perennial atopic rhinitis are initially induced by hypersensitivity to inhaled exogenous allergens especially to the following substances house dust animal danders feathers environmental and poultry dusts and fungi besides sensitization to the allergenic plant pollens Patients with perennial atopic rhinitis in the Bydgoszcz region are hypersensitive to the same allergens as patients from other parts of Poland (Bystrzanowska et al 1973 Chyrek Borowska et al 1970) Evidently these allergens have a decidedly cosmopolitan character and sensitize atopic indi viduals throughout the world regardless of geographic or climatic zone (Romański & Jonak 1969) *Hypersensitivity to inhaled* allergens often induces attacks of bronchial asthma, the atopic form of which throughout the world is due to the same allergens mentioned above (Romański, 1976) Atopic bronchial athma is also dependent on systemic factors identical with those in patients with perennial atopic rhinitis

3 In the light of current knowledge every thing seems to point to bronchial hyperreactivity as a factor predisposing to bronchial asthma in some subjects. This characteristic stigma of bronchial asthma may be either congenital or acquired At present inhalation

Table IV *Age of the examined patients at onset of the first symptoms of perennial a rhinitis versus bronchial reactivity*

		Number of patients								
		without bronchial hyper reactivity (n=122)		with bronchial hyperreactivity TH (total hyperreactivity)=115						
No	Age group	(−)	% n	+ n=46	% n	++ n=57	%	+++ n=12	%	Total (TH 115
1	0–4	2	1 64	1	2 17	8	14 04	1	8.33	10
2	5–9	7	5 74	4	8 70		3.51	3	25 0	9
3	10–14	15	12.29	5	10 87	10	17.54		16 67	17
4	15–19	23	18 86	12	26.09	10	17.54	–	–	22
5	20–24	25	20 49	4	8.70	4	7 0.	4	33.34	1
6	25–29	19	15 57	7	15.22	3	5.26	1	8 33	11
7	30–34	14	11 47	4	8 70	5	8 77	–	–	9
8	35–39	6	4 92	5	10 87	11	19 30	–	–	16
9	40–44	8	6.56		4.34	4	7 02	–	–	6
10	45–49	2	1 64	1	2.17	–	–	1	8.33	
11	50–54	1	0 82	1	2.17	–	–	–	–	
Total		122	100 0	46	100.0	57	100 0	1	100 0	115
% of examined patients (N=237)		51 48		19 41		4 05		5 06		48 52

tests of sensitivity of bronchi to physical exercise and histamine in 106 patients with atopic bronchial asthma and 128 healthy active sportsmen in whom repeated specialist medical examinations showed no abnormalities (Dziedziczko 1976) Bronchospasm was served in all cases of bronchial asthma an 6 5% of healthy non-atopic subjects, 8 7% of healthy with a history of hered atopy

Table V *Heredity and bronchial reactivity in the patients population with perennial al rhinitis*

		Number of patients									
				with hereditary encumbrance TH (total hyperreactivity)=163							
No	Bronchial reactivity degree	without hereditary encumbrance (n=74)	%	in one member of the family (=62)	% n	in many members of the family (n=101)	%	Total (TH=163)	% TH	All patients	% of exam patien (V=…
1	(−)	59	79 73	26	41 93	37	36 63	63	38.65	1.2	51 48
	+	7	9 46	13	20 97	6	.5 74	39	.3 93	46	19 41
3	++	7	9 46	17	27 4.	33	32.68	50	30 67	57	24 05
4	+++	1	1 35	6	9 68	5	4 95	11	6.75	1	5 06
Total		74	100 0	6.	100 0	101	100 0	163	100 0	.37	100 0
Patients with bronchial hyper reactivity (% n)		15	20 27	36	58.06	64	63.37	100	61.35	115	48.5

pa- ts	% of examined patients (N = 37)
	31.22
	46.42
	22.36
	100.0
0	

clearly show that although bronchial hyperreactivity may sometimes be detected in apparently healthy subjects it is usually associated with a congenital predisposition to allergic diseases i.e. atopy

Our observations on the incidence of bronchial hyperreactivity in patients with perennial atopic rhinitis agree with the results of other investigators who used different study methods and criteria, using acetylcholine (Ionescu 1973 Molner et al 1967) metacholine (Grossman & Putnam, 1975) as well as histamine (Goldman & Owczarenko 1970) yet obtained the same results as we did

From the practical point of view it is of importance to know the extent to which bronchial hyperreactivity present in patients with perennial atopic rhinitis can induce the physician to take measures aimed at sparing these patients from the occurrence of atopic bronchial asthma.

This information is of clinical importance because: (*a*) it reminds otolaryngologists that perennial atopic rhinitis is a systemic symptom of atopic diathesis (*b*) it calls attention to the fact that since the same allergens are responsible for symptoms of allergic rhinitis and bronchial asthma, appropriate measures such as removal of allergens from the patient's surroundings or early specific desensitizing therapy can effectively control the symptoms of perennial atopic rhinitis and prevent evolution of the disease Moreover the allergic reaction in the bronchi can be prevented In addition atopic subjects especially patients with concomitant bronchial hyperreactivity can be advised to choose as far as possible work at which they will be spared contact with common allergens and nonspecific irritants of the respiratory system

On the basis of these findings, perennial atopic rhinitis deserves a new look. Although it is an ubiquitous disease in everyday otolaryngologic practice sight is often lost of the fact that perennial atopic rhinitis is very often the first alarm signal warning agnst the possibility of developing bronchial asthma, a much more serious disease with unquestioned social impact.

CONCLUSIONS

The following general conclusions may be formulated.

(1) Perennial atopic rhinitis and atopic bronchial asthma are two manifestations of atopic disease caused by the same allergens particularly house dust, animal danders feathers and some environmental dusts besides dusts from poultry and fungi

(2) The course of perennial atopic rhinitis may be divided into the following consecutive stages. paroxysmal catarrhal vasodilative chronic edema, hyperplastic and polypous The disease progresses in untreated patients

(3) In one-half of cases perennial atopic rhinitis can be diagnosed objectively on the basis of exercise tests and the histamine test in bronchial hyperreactivity The patients are prone to develop bronchial asthma.

Mathematical analysis of the spirographic data shows statistically significant correlation between bronchial hyperreactivity and stage of nasal catarrh, age of patient and heredity

(4) Appropriate early causal treatment (elimination of the allergen or specific desensitization) in patients with perennial atopic rhinitis cause speedy regression of symptoms

Table VI *The clinical course of the disease and bronchial reactivity*

		Number of patients without bronchial hyper reactivity (n = 122)		with bronchial hyperreactivity TH (total hyperreactivity) = 115							
No	Clinical course	(−)	% n	+ n = 46	% n	++ n = 57	% n	+++ n = 12	% n	Total (TH 115)	[illegible]
1	Mild	48	39 45	15	32.61	11	19 30	–	–	26	
2	Moderately severe	51	41.80	21	45 65	30	52.63	8	66.67	59	
3	Severe	23	18 86	10	21 74	16	28 07	4	33.33	30	
All patients		1_2	100.0	46	100 0	57	100 0	12	100 0	115	1
% of examined patients (N = _37)		51 48		19 41		?4 05		5 06		48.52	

tests with histamine or acetylcholine and exercise tests enable us to detect the state of bronchial hyperreactivity in bronchial asthma patients in the period between attacks in subjects without clinical symptoms of the disease in a state of asthma readiness or preasthmatic state

Our observations on the exercise and histamine tests in perennial atopic rhinitis patients are highly interesting A total of 115 patients (48 52% of the population studied) had symptoms of bronchial hyperreactivity identical with those in bronchial asthma patients (Tables II–VI) Mathematical analysis of our data showed statistically significant correlation between bronchial hyperreactivity and age of patients (Table III) heredity (Table V) and stage of advancement of the disease Bronchial hyperreactivity was not significantly correlated with sex age at onset of first clinical symptoms of the disease or intensity of symptoms (Tables IV and VI) or type of work and exposure to respiratory tract irritants during work

Everyday clinical observations show that in higher age groups and after many years duration of perennial atopic rhinitis the disease evolves in most patients It may be concluded that the longer duration of perennial atopic rhinitis and the greater changes in the mucous membrane of the nose and paranasal sinuses the greater the degree of bronchial hyperre tivity

In our material bronchial hyperreactiv type of occupation and exposure to respira irritants during work were not correlated contrast to our previous results (Dziedzic et al 1977) of a study on the workers c chemical plant in Bydgoszcz where conti contact with respiratory irritants elimin workers with bronchial hyperreactivity (l rate of absence from work) who soon l for a different job An unfavorable amb microclimate at work can influence evolu of the disease

Another interesting observation was m when studying bronchial hyperreactivity u the exercise and histamine tests in patie with rhinitis vasomotorica nonallergica v 22 2% positive results and in adults with fever in 58% (Dziedziczko & Gniazdow 1977a) in pupils and students with hay fe in 55% (Dziedziczko & Gniazdowski 19 and in subglottic laryngitis in 64 6% (Gn dowski & Dziedziczko 1977) in atopic tients with nasal polyps in 53 3% and in n atopic patients with nasal polyps in 52. (Romański et al 1978) Moreover bronc hyperreactivity was observed in 6.51% healthy non-atopic subjects and in 8 37% healthy subjects with a history of heredit atopy (Dziedziczko

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of rhinitis and prevent local complications (nose and paranasal sinuses) besides effectively preventing future development of bronchial asthma in patients with bronchial hyper reactivity

ZUSAMMENFASSUNG

Man hat die Proben bei 237 Kranken unternommen um die ätiologischen Faktoren und die Häufigkeit des Auftretens der Bronchialüberempfindlichkeit als „Stigma des Bronchialasthmas bei allergischem ganzjährigem Nasenkatarrh zu bestimmen Bei allen Patienten wurden ausführliche laryngologische allergische und spirographische Untersuchungen im Ruhezustand (CV VEMS DET IS) nach einer Anstrengung (PWC_{170}) und nach der Einatmung des Histamins durchgeführt Daraus ergeben sich folgende Schlußfolgerungen 1 Allergischer ganzjähriger Nasenkatarrh und allergisches Bronchialasthma gehören zu den zwei Symptomen der atopischen Krankheit. Sie ist durch dieselben Allergene wie Staub Federn Ober und Tierhaut, Schimmelpilze hervorgerufen. 2. Im Krankheitsverlauf des allergischen, ganzjährigen Nasenkatarrhs sind folgende Entwicklungsstadien zu nennen Krankheitsanfall Katarrh Gefäßeinbuße langwierige Schwellung, Wachstum, Polyp 3 In der Hälfte des Krankenguts mit den Fällen des Nasenkatarrhs kommt zum Vorschein Symptom Bronchialüberempfindlichkeit, was durch einfache Anstrengungsproben und Provokationsversuch der Einatmung des Histamins objektiv nachzuweisen ist Diese Kranken sind besonders anfällig fur Bronchialasthma. Die statistischen Angaben der spirographischen Proben zeigen daß es statistisch eine Wechselbeziehung zwischen der Bronchialuberempfindlichkeit und dem Nasenkatarrhfortschritt, dem Krankenalter und der erblichen Belastung gibt 4 Rechtzeitige Unternehmung der Ursachentherapie (die Beseitigung der Allergene die Entsensibilisierung) kann zur Zurückbildung des allergischen Katarrhs beitragen sowie auch zur Vorbeugung der nabwendbaren Veranderung im Nasenschleimhaut und Nasennebenhohle fuhren Die Therapie kann auch diese Kranken vor dem Bronchialasthma schutzen bei denen schon die Bronchialuberempfindlichkeit aufgetreten ist

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[illegible]		Day				[illegible]
Time		morning	afternoon	night		[illegible]
Symptom	Sneezing	4	1	3		[illegible]
	Nasal secretion	3	0	3		[illegible]
	Nasal blockage	+	#	#		[illegible] (#) [illegible] (#) [illegible] (+) [illegible] (−)
	Olfactory abnormality	+	—	#		[illegible] (#) [illegible] (#) [illegible] (+) [illegible] (−)
	Daily life	#	—	#		[illegible] (#) [illegible] (+) [illegible] (#) and (+) — (#) [illegible] (−)
	Others	[illegible]		headache		[illegible]
Cause	Cause of attack	[illegible]		[illegible]		[illegible]
	Place where attack occurred	[illegible]	[illegible]	[illegible]		[illegible]
	Others	[illegible]				[illegible]
Therapy daily dose	Spraying	2	2	2	2	8 sprays/day ([illegible])
	Others	[illegible]				[illegible]
Other symptoms noticed		[illegible]				[illegible]

Fig. 1 Nasal allergy diary

30° and 0° from the horizontal plane. NR was recorded when the nasal airflow was 0.25 l/sec. NR at an angle of 90° was determined first and followed by at angles of 60°, 30° and 0°. A subject was kept in a new position for 1 min at each angle before NR was determined. Increase or decrease of NR was calculated at each angle in comparison with the NR of the 90° angle. Postural variations of NR were tested at the end of the first, the second and the third week.

RESULT

Normal subjects

The postural variations of NR in normal subjects are shown in Fig. 4. The mean value of NR gradually increased from that at the angle of 90° to the angle of 0°. However, it was a very slight change.

Patients with allergic rhinitis

The postural variations of NR in patients with allergic rhinitis were remarkable. Fig. 5 shows the postural variations of NR which were observed at the end of the first, the second and the third week. The improvement in postural variation of NR was induced by Beclomethasone Dipropionate therapy. However, 2 out of 15 patients did not show any remarkable postural variation, even though they had nasal symptoms of allergic rhinitis.

All patients showed an improvement in nasal symptoms of allergic rhinitis following Beclomethasone Dipropionate therapy, though not in every patient was there always a co-

[illegible]	#	+		
[illegible]	[illegible]	9 – 5	[illegible] 4	0
[illegible]	[illegible]	9 – 5	[illegible] 4	0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	3	2	1	0

Fig. 2 Nasal symptom scoring method.

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POSTURAL VARIATIONS IN NASAL RESISTANCE AND SYMPTOMATOLOGY IN ALLERGIC RHINITIS

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(Received November 9 1978)

Abstract Variations in nasal resistance (NR) which was induced by positional change of the head were examined in 15 normal subjects and 15 patients with allergic rhinitis Nasal symptoms were also observed in 15 patients and nasal symptom scores were calculated Concerning the postural variations of NR, *patients with allergic rhinitis* showed much more remarkable change than normal subjects Nasal symptom scores of all patients decreased after nasal insufflation of Beclomethasone Dipropionate for 2 weeks Coincidence between improvement in postural variations of NR and that of nasal symptom score was not always seen in each patient. However as a mean value of 15 patients postural variations of NR improved after nasal insufflation of Beclomethasone Dipropionate

Nasal resistance (NR) is not always constant even in normal subjects It is influenced by several factors i e positional changes of the head breath holding rebreathing hyperventilation exercise temperature and humidity (Seebohm & Hamilton 1958 Salman et al 1971 Hasegawa et al 1976 Dallimore & Eccles 1977) Kayser (1895) and Heetderks (1927) reported that lying on one side caused obstruction of the lower passage of the nose Connor et al (1957) and Hamberger (1961) reported that nasal congestion was caused by interrupted sympathetic nerve impulses Malm (1973) demonstrated that stimulation of sympathetic nerve fibers caused the decrease of venous blood flow and increase of nasal patency in the nose

Concerning the postural variations of NR Rundcrantz (1969) reported that the different response was seen between in normal subjects and in patients suffering from allergic rhinitis Normal subjects showed a slight increase of NR in horizontal recumbent position while in patient with allergic rhinitis NR was about three times higher in the horizontal recumbent position than in the sitting position

The aim of this study was to learn whether postural variations of NR are related to the severity of the symptoms of allergic rhinitis and whether these change according to the improvement of nasal symptoms following Beclomethasone Dipropionate therapy

SUBJECTS AND METHODS

Fifteen normal subjects (8 males 24-31 years of age 7 females 19-28 years of age) and 15 patients with allergic rhinitis (7 males 18-6[illegible] years of age 8 females 20-48 years of age) were examined All of the patients had perennial allergic rhinitis that was caused by house dust All the patients were treated only with nasal insufflation of Beclomethasone Dipropionate aerosol 50 μg for each nostril four times a day (total dose of a day is 400 μg)

They recorded their nasal symptoms of allergic rhinitis in a nasal allergy diary in 3 weeks During the first week they were not treated In the second and the third week they themselves insufflated Beclomethasone Dipropionate into each nostril The nasal allergy diary was filled in by the patients as shown in Fig 1 Nasal symptom scores were calculated according to the nasal symptom scoring method (Fig 2)

NR was determined by the posterior method of rhinomanometry (Fig 3) Postural variations of NR were observed at angles 90° 60°

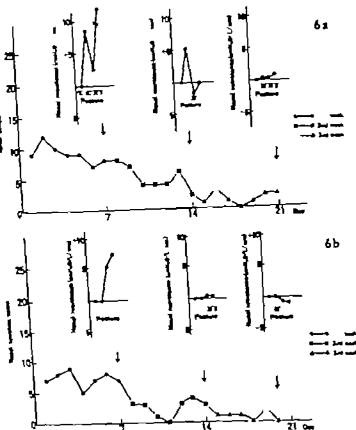


Fig 6 Improvement in postural variations of nasal resistance and nasal symptom score are coincident. ( ) Case 1 (female age 49) (b) Case (female age 20)

hypotony due to a preponderance of the parasympathetic autonomous system is presumed to be the cause

In our study 2 out of 15 patients did not show any remarkable postural variations of NR We cannot understand why a positional change of the head failed to cause an increase in NR in these patients They were no different from the other patients as regards symptoms the duration of the disease or allergen

All the patients demonstrated an improvement in nasal symptom scores following Beclomethasone Dipropionate therapy Beclomethasone Dipropionate is a synthetic corticosteroid which differs from ordinary corticosteroids in several aspects As one characteristic property this steroid has no significant systemic activity when it is used locally A daily dose of 400 μg Beclomethasone Dipropionate did not affect the plasma cortisol and was effective in about 75% of patients suffering from perennial rhinitis (Gibson et al 1974 Hansen & Mygind 1974) Moreover according to Mygind et al. (1976) it was also effective in about 80% of patients suffering from moderate to severe nasal polyposis

In our study a coincidence between the improvement in postural variations and nasal symptom scores was not always seen in each patient. The cause of this discrepancy in the two examinations cannot be indicated with certainty However as a mean value of the 15 patients postural variations of NR were improved more in the second week than in the first week, and more in the third week than in the second.

Beclomethasone Dipropionate brought about an improvement in postural variations of NR The pathological mechanism which

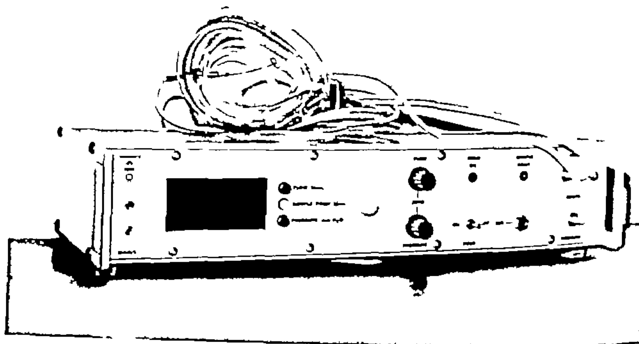


Fig 3 Nasal resistance meter NRI (mercury)

incidence between the improvement in nasal symptoms and the improvement in postural variation of NR

DISCUSSION

Postural variations of NR in patients with allergic rhinitis were first reported by Rundcrantz in 1964 In 1969 he also reported the fact that this phenomenon was recognized not only in allergic rhinitis but also during a common cold This pathological mechanisms has not yet been elucidated However vascular

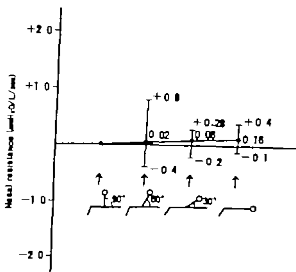


Fig 4 Postural variations of nasal resistance in 15 normal subject

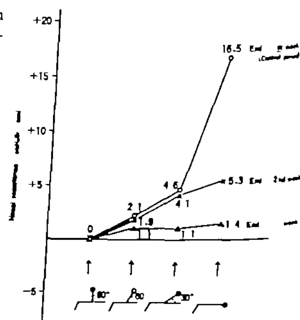


Fig 5 Improvement in postural variations of nasal resistance following administration of Beclomethasone Dipropionat In patients (15) with allergic rhinitis.

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ENDOSCOPIC EXAMINATION OF THE NASOPHARYNX

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Abstract Endoscopic examination of the nasopharynx is simple and rapid procedure The inspection can be made through the nose. This method is recommended for screening. If abnormalities of the nasopharynx are disclosed and are to be studied in detail and particularly if such abnormalities are to be documented photographically it is necessary to inspect them through the oropharynx while the soft palat is drawn forward. It is possible under direct vision to tak representative biopsy specimens, and it is easy to take photographs for the purpose of documentation, comparison and education. This procedure can to great extent replace diagnostic excision of tissue from the nasopharynx under general anaesthesia. Especially with view to early diagnosis of malignant disease of the nasopharynx it is important to extend the use of this method, which can be performed with little discomfort to the patient.

Owing to the situation of this region Inspection of the nasopharynx is is often difficult. The need for a method of examination which would be more effective than the conventional study using a postnasal mirror was felt relatively early The first nasopharyngoscope for inspection through the oropharynx was devised by Hays (1909) During the last decade or so the method has gained ground because of the considerable technical advances which have occurred (Berci et al 1967 Butter 1976 Ward et al 1976) Owing to the development of Hopkins optics and cold light it is now possible to obtain an excellent view of the nasopharynx to secure biopsy specimens under direct vision and to take colour photographs for the purpose of documentation and education.

Technique

Equipment

The Storz equipment used (Fig. 1) comprises 70° and 120° Hopkins optics both 4 mm in diameter and with a field of vision of 90° can also be used for rhinoscopy sinoscopy and transconioscopy) a Storz uvula retractor for these optics and a 90° pharyngo-laryngoscope with a visual field of 60° The palate retractor employed is usually a length of rubber tubing fastened to a metal plate The light source is a combined cold-light generator and flash unit For photographic exposures a Stortz lightweight camera with a motorized film transport and an objective with a focal length of 90 mm weight 320 g, is used

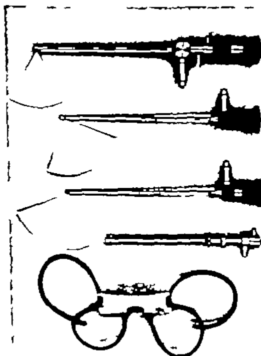


Fig 1

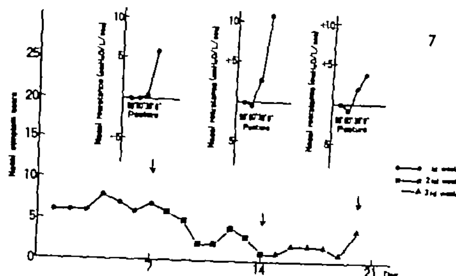


Fig 7 Case 3 (male, age ?) Postural variations in nasal resistance and improvement in nasal symptom score are not coincident

causes postural variations in patients suffering from allergic rhinitis is presumably explained by the venous stasis related to allergic reaction If this is so the anti-inflammatory effect of Beclomethasone Dipropionate may have reduced the vascular hypotony

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ZUSAMMENFASSUNG

Die durch die Änderung der Kopflage hervorgerufenen Variationen der nasalen Luftdurchlässigkeit (NLD) wurde bei 15 gesunden Probanden und bei 15 Patienten mit allergischer Rhinitis untersucht. Dabei wurden bei 15 Patienten auch die nasalen Symptome kontrolliert und die nasal symptom scores kalkuliert. Bezüglich der postūralen Variationen der NLD zeigten die Patienten mit allergischer Rhinitis viel deutlichere Änderungen als normale Personen Die „nasal symptom scores" verminderten sich bei allen Patienten durch die 2wöchige nasale Anwendung von Beclometason dipropionat Die Verbesserung der posturalen Änderungen der NLD geht bei einigen Patienten nicht immer parallel mit denjenigen der „nasal symptom scores" Jedoch haben sich die postural bedingten Variationen der NLD—als einen Mittelwert an 15 Patienten gesehen—durch die nasale Anwendung des Beclometason dipropionats gebessert

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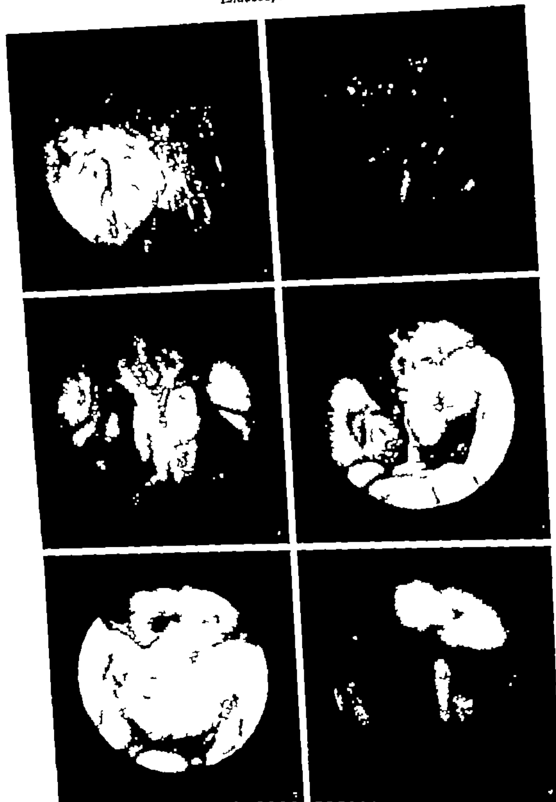




Fig 2

Anaesthesia

For pernasal screening examination of the nasopharynx it is fully sufficient to apply topical anaesthesia to the nasal mucosa When the inspection is performed through the oropharynx it is also necessary to spray a surface anaesthetic on the base of the tongue and the posterior wall of the pharynx including the nasopharynx



Fig 3

Method

Pernasal nasopharyngoscopy is performed with 70° optics introduced along the floor of the nasal cavity The posterior margin of the septum is identified and the opposite side of the nasopharynx is then inspected where excellent view of the region around the op ing of the Eustachian tube can be obtain (Fig 4) This method is best as a screen procedure and does not cause any discomf to the patient

In the presence of abnormalities of the na pharynx which are to be subjected to clo examination and particularly if the findi are to be documented photographically it necessary to perform the inspection throu the oropharynx which requires that the s palate is pulled forward during the exami tion When 70 and 120 optics are used t uvula retractor can be applied However better view is obtained by pulling the s palate forward by reins (Fig ?) By tl method rubber catheters are introduc through the two nostrils and led forwa through the mouth The soft palate is th pulled forward and the reins can be fix to a metal plate resting on the upper lip Tl

use of the 90° optics gives an appreciably larger magnification than the 70° and 170° optics because of the narrower field of vision and it is therefore particularly well suited for the inspection of the choanae, the lower part of the choanae which it is very difficult to inspect by means of a mirror is also distinctly seen (Figs 6 and 7)

Biopsy

By means of a straight biopsy forceps introduced through one nostril and the optics through the other or by inspection through oropharynx biopsy specimens can be taken under direct vision (Fig 3) A curved biopsy forceps introduced through the mouth may so be used

Endoscopic Photographs

ormal appearance (Figs 4–5)

ig 4 shows the appearance of the normal ght opening of the Eustachian tube photographed through 70° optics introduced through he left side of the nose Similarly Fig 5 hows the normal nasopharynx (In the endoscope the picture is viewed upside down t is here turned 180° so that the right side of the nasopharynx is seen to the left in the photo) In the upper part of the posterior wall of the nasopharynx remnants of partially trophied adenoids are visible Note the excellent view of the openings of the Eustachian tubes

P thological onditions of the posterior part of the nasal cavity (Figs 6–8)

Such conditions are often best inspected by nasopharyngoscopy In Fig 6 papillomata are sible on both sides of the septum whereas the choanae are otherwise perfectly normal

Nasal polypi may often be seen in the choanae As surgical removal of polyps from the posterior part of the nose is difficult it will often be an advantage to do this under direct vision (Fig. 7)

The solitary non-allergic choanal polypus arises from the maxillary sinus with a thin pedicle It is seen to protrude into the nasopharynx In extreme cases it may hang down into the oropharynx (Fig 8)

Choanal atresia (Fig 9)

Both unilateral and bilateral choanal atresia can easily be visualized through the endoscope even in infants During surgical treatment, it is of importance that it can be seen directly how much the ostium is dilated and that the proper position of the inserted tube can be checked

Tumours (Figs 10–12)

Endoscopic examination of the nasopharynx is of particular importance in the diagnosis of tumours Nasopharyngoscopy is also a valuable aid in early detection of recurrence after surgical treatment or radiotherapy

Fig. 10 shows a prominent malignant lymphoma of the posterior wall of the nasopharynx

Fig. 11 shows the recurrence of a squamous-cell carcinoma in the upper part of the nasopharynx 12 months after radiotherapy

A juvenile angiofibroma arising from the top of the left side of the nasopharynx partly covered by fibrinous materials and small blood clots is seen in Fig. 12

Other diseases of the nasopharynx (Figs 13–15)

Fig. 13 shows large adenoid vegetations and ample amounts of mucopus in a 6-year-old girl with adenoiditis

A large smooth swelling of the posterior wall of the nasopharynx is shown in Fig 14 It appeared to be a cyst (Thornwaldt s cyst)

Osler s disease is manifested by frequent haemorrhages from ectatic small vessels principally from the nasal mucosa. Fig 15 shows telangiectases in a patient with a moderate tendency to bleeding and multiple telangiectic lesions on the lips tongue and face In addition considerable swelling is seen of the posterior ends of the inferior turbinates which ha e a cyanotic hue






12


13


14


15

use of the 90° optics gives an appreciably larger magnification than the 70° and 120° optics because of the narrower field of vision and it is therefore particularly well suited for the inspection of the choanae the lower part of the choanae which it is very difficult to inspect by means of a mirror is also distinctly seen (Figs 6 and 7)

Biopsy

(By means of a straight biopsy forceps introduced through one nostril and the optics through the other or by inspection through oropharynx biopsy specimens can be taken under direct vision (Fig. 3) A curved biopsy forceps introduced through the mouth may also be used

Endoscopic Photographs

Normal appearance (Figs 4–5)

Fig 4 shows the appearance of the normal right opening of the Eustachian tube photographed through 70° optics introduced through the left side of the nose Similarly Fig. 5 shows the normal nasopharynx (In the endoscope the picture is viewed upside down It is here turned 180° so that the right side of the nasopharynx is seen to the left in the photo) In the upper part of the posterior wall of the nasopharynx remnants of partially atrophied adenoids are visible Note the excellent view of the openings of the Eustachian tubes

Pathological conditions of the posterior part of the nasal cavity (Figs 6–8)

Such conditions are often best inspected by nasopharyngoscopy In Fig 6 papillomata are visible on both sides of the septum whereas the choanae are otherwise perfectly normal

Nasal polyps may often be seen in the choanae As surgical removal of polypi from the posterior part of the nose is difficult it will often be an advantage to do this under direct vision (Fig. 7)

The solitary non-allergic choanal polypus arises from the maxillary sinus with a thin pedicle It is seen to protrude into the nasopharynx In extreme cases it may hang down into the oropharynx (Fig 8)

Choanal atresia (Fig 9)

Both unilateral and bilateral choanal atresia can easily be visualized through the endoscope even in infants During surgical treatment it is of importance that it can be seen directly how much the ostium is dilated and that the proper position of the inserted tube can be checked.

Tumours (Figs 10–12)

Endoscopic examination of the nasopharynx is of particular importance in the diagnosis of tumours Nasopharyngoscopy is also a valuable aid in early detection of recurrence after surgical treatment or radiotherapy

Fig 10 shows a prominent malignant lymphoma of the posterior wall of the nasopharynx

Fig 11 shows the recurrence of a squamous-cell carcinoma in the upper part of the nasopharynx 12 months after radiotherapy

A juvenile angiofibroma arising from the top of the left side of the nasopharynx partly covered by fibrinous materials and small blood clots is seen in Fig. 12.

Other diseases of the nasopharynx (Figs 13–15)

Fig. 13 shows large adenoid vegetations and ample amounts of mucopus in a 6-year-old girl with adenoiditis

A large smooth swelling of the posterior wall of the nasopharynx is shown in Fig. 14 It appeared to be a cyst (Thornwaldt's cyst)

Osler's disease is manifested by frequent haemorrhages from ectatic small vessels principally from the nasal mucosa. Fig. 15 shows telangiectases in a patient with a moderate tendency to bleeding and multiple telangiectic lesions on the lips tongue and face In addition considerable swelling is seen of the posterior ends of the inferior turbinates which have a cyanotic hue

DISCUSSION

Diseases localized in the nasopharynx are difficult to diagnose and their symptoms are often uncharacteristic Conventional indirect nasopharyngoscopy is difficult to perform and very often even an experienced examiner will manage to catch only a brief glimpse of the region which is quite insufficient to exclude the presence of a neoplasm It has been shown that 40% of the patients in whom malignant tumours of the nasopharynx are diagnosed have had symptoms for more than 6 months and 20% for more than 12 months and that they have often applied for otological attention for their symptoms (Bertelsen et al 1975) It is therefore evident that there is a very great need for a better method of examination Pernasal nasopharyngoscopy with optics (by some authors termed salpingoscopy) is a reasonably good and rapid screening method without discomforts to the patient Inspection through the oropharynx with the soft palate drawn forward gives a good view of the region with only modest discomfort to the patient The method provides a possiblity of securing representative biopsy specimens and of taking photographs for the purpose of documentation comparison and education By this technique it is possible to avoid many diagnostic excisions from the nasopharynx under general anaesthesia.

ACKNOWLEDGMENT

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RHINOSCOPICAL FINDINGS IN NICKEL WORKERS WITH SPECIAL EMPHASIS ON THE INFLUENCE OF NICKEL EXPOSURE AND SMOKING HABITS

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Abstract. Rhinoscopy and X-ray examination were performed on 318 nickel workers and 57 controls, to study the significance of these methods in detecting cancerous and precancerous mucosal changes. The clinical and radiological findings were compared with histopathological data and mucosal nickel concentrations determined in nasal biopsy material from the middle turbinate, with duration of nickel exposure and with tobacco smoking habits. The nickel-exposed subjects had statistically significantly more pathological changes (43%) than the controls (26%), $(0.01 < P < 0.02)$, mainly due to differences in the frequency of hyperplastic rhinitis. Thirteen nickel workers (4%) had nasal polyps. Two of these cases, both employed at the nickel refinery for 28 years, appeared to have nasal carcinoma, according to histological examination. No distinct association was established between rhinoscopical findings and epithelial dysplasia found by histological examination. The explanatory values for the rhinoscopical findings of different factors, such as working category, age, duration of nickel exposure, grams tobacco smoked per week, and nickel content of nasal mucosa, were evaluated by applying stepwise multiple regression analysis. Number of years from first employment at the nickel refinery and tobacco consumption were the only explanatory factors that showed statistically significant correlation to the rhinoscopical findings. The radiological examination revealed few characteristic findings. Chemical analysis of cigarettes handrolled by nickel workers showed high nickel concentrations compared with non-contaminated cigarettes.

Reports on increased risk of respiratory tract cancers in nickel workers have been reviewed in comprehensive monographs and surveys (Sunderman et al. 1975, IARC report 1976, NIOSH report 1977). Sunderman (1977) has at March 1977 collected 477 nickel-induced lung cancer cases and 143 nasal cancers from the literature.

The particularly high risk for nasal carcinoma in nickel workers has been emphasized in a report from a Norwegian nickel refinery (Pedersen et al. 1973). Histological examinations of biopsy specimens from the nasal mucosa in these workers have revealed dysplasia of the surface epithelium (Torjussen & Solberg, 1976, 1978). Tobacco smoking seems to be another factor that influences the histopathology of the nasal mucosal epithelium in both nickel workers and controls (Torjussen & Solberg, in press, 1978).

The present investigation was undertaken to study the significance of rhinoscopy and X-ray examination of paranasal sinuses in detecting cancerous and precancerous mucosal changes in nickel workers. Our rhinoscopical and X-ray findings have been compared with histopathological and chemical data obtained on nasal biopsies from the same subjects (Torjussen & Solberg, in press, 1978; Torjussen et al. 1978), to duration of nickel exposure, and to tobacco smoking habits. In this connection the nickel content in cigarettes was determined.

MATERIALS AND METHODS

The nickel refining process

The raw material for the nickel production at the plant is converter matte (approximately 50% nickel, 30% copper, 20% sulphur and some trace metals), which is refined through several processes including crushing, roasting, smelting and electrolysis. Workers involved in the first three processes mentioned are exposed mainly to dry dust containing nickel compounds which are almost insoluble in water (nickel subsulphide and oxide). The

Table I *Primary selected (370) and attending number of nickel workers (318) and controls (57), mean age and number of years from the first nickel exposure distributed by category of work*

Work category and category of subjects	No of workers primarily selected	No of attendants n (%)	Mean age (yrs) (range)	Mean time (yrs) from first nickel exposure (range)
Roasting/smelting	1_3	97 (79%)	50 9 (4–70)	19 9 (8–40)
Electrolysis	163	144 (89%)	50 9 (_8–69)	20 9 (8–44)
Non-process work	84	77 (9 %)	5_1 (30–67)	_3 2 (8–41)
Nickel workers	370 (100%)	318 (86%)	51_ (4–70)	21_ (8–44)
Controls		57	50 7 (29–67)	–

electrolysis workers are *mostly exposed to* aerosols of water soluble composites (nickel sulphate and chloride) *Non process workers* not directly involved in the above mentioned processes *are generally exposed to miscel* laneous nickel compounds

The nickel exposed group

Subjects employed at least 8 years at the plant and working with crushing roasting smelting *and electrolysis were all selected for the inves* tigation An additional 20% of the non process *workers employed at least 8 years at the re* finery were selected at random by a computer *This primary selected material comprised* 370 individuals Some 318 subjects (86% 316 *men and 2 women) consented to attend the* investigation 37 persons (10%) refused and *15 (4%) were ill and unable to participate* All the participants were allocated to one of *three categories of work where they were* employed October 1st 1976 (Table I) and the *investigation was completed during the sub*sequent 3 months Forty six of the participants *had been employed in more than one work* category but only 28 of these were according *to this procedure allocated to the work cate* gory with the shortest employment Duration *of nickel exposure is reckoned as years from* first employment at the plant

The control group

This group consisted of 57 male volunteer patients at the Central County Hospital Kris *tiansand matched by age with the nickel* exposed group and selected consecutively *during the last 3 months of the year 1977* (Table I) Subjects with former or present *employment in the nickel industry* with ex isting diseases of the nose or paranasal sinuses or with general systemic diseases were ex cluded Twenty two of the patients were re *gistered at the ear nose and throat depart*ment and 35 at the surgical department of the hospital

Previous history

Previous nasal diseases and subjective nasal complaints occupational history in h nickel exposure and smoking habits were eval uated by a questionnaire and an interview Subjects who had stopped smoking less than one year before the start of the investigation were registered as smokers

Rhinoscopical examination

The examination was done without access to the personal or occupational data and with the subjects lying horizontally Cases of acute nasal inflammation were not evaluated until cured *On the basis of the rhinoscopical find*ings the cases were given scores according to *the following characteristics*

0 no abnormalities
1 hyperplastic rhinitis i e marked swelling of the turbinates p middle one *still* , after

Table II *Subjective nasal complaints in nickel workers (318) and in controls (57)*

Subject category	N of subject	No. of subjects without nasal complaints (%)	No of subjects with nasal complaints (%)	Types of nasal complaints: Obstruction (%)	Recurrent epistaxis (%)	Allergy (%)	Recurrent long-lasting secretion, chron sinusitis (%)
Nickel workers	318	4_ (76)	76 (24)	54 (17)	18 (6)	6 (2)	19 (6)
Controls	57	43 (75)	14 (25)	8 (14)	1 (2)	1 (2)	5 (9)
Σ	375	285 (76)	90 (24)	6_ (17)	19 (5)	7 (2)	4 (6)

of adrenalin to the nasal mucous membrane (Fig. ?)

2. polypoid mucosal surface with or without single or multiple polyps

3 more localized swelling or thickening of the mucous membrane giving suspicion of a neoplastic tumorous lesion

X-ray examination

Radiologic examination of the paranasal sinuses included occipito-mental occipito-frontal and lateral projections These were examined independently by a radiologist and the author without access to clinical or epidemiological information. The findings were grouped according to either of the following characteristics

No abnormalities also including insignificant thickening of the maxillary sinus mucosa (i e less than ? mm)

Abnormal finding including all positive X-ray findings related to the paranasal sinuses and not mentioned above

The grouping by both examiners was identical in 349 of the 375 cases The authors results were used in the further study

Histological examination

Biopsies were taken from the anterior curvature of either of the two middle turbinates immersed in 3% glutaraldehyde in 0 1 M cacodylate buffer at pH 7 4 embedded in Epon cut into semithin sections and stained with toluidine blue The results of the histopathological gradings of these specimens are reported in detail elsewhere (Torjussen & Solberg in press 1978) and are only used in the present study for comparison with the clinical findings (Table VI)

Chemical examination

Nickel concentrations in nasal mucosa (μg/100 g wet weight) were determined by atomic absorption spectrometry (Torjussen et al 1977) The results are reported elsewhere (Torjussen et al 1978) and are only used in this study for the multiple regression analysis (see below)

Nickel contents in tobacco and cigarette paper were analysed by the same technique as for the above-mentioned tissue analyses (Torjussen et al 1977) as follow

1 Ten samples (mean weight 0 3 g) of Norwegian-produced cigarette tobacco (Tiedemanns gul) and 10 cigarette papers were analysed separately The utmost care was taken to avoid nickel contamination.

Samples (mean weight 0 9 g) of the same tobacco and cigarette papers were distributed among 20 randomly selected process workers on duty 10 from the roasting/smelting department and 10 from the electrolytic department all heavy smokers. Each worker was asked to roll one cigarette. The tobacco and the cigarette papers were analysed separately

3 The tobacco and cigarette papers from 10 U S produced cigarettes (Pall Mall) were analysed separately for nickel content

Table III *Rhinoscopical findings in nickel workers distributed by category of work and in controls*

Work category and category of subjects	*No of subjects*	Rhinoscopical findings: No abnormalities *n* (%)	Hyperplastic rhinitis *n* (%)	Polypoid mucosa, polyps tumour *n* (%)
Roasting/smelting	97	5 (54)	39 (40)	6 (6)
Electrolysis	144	83 (58)	55 (38)	6 (4)
Non-process work	77	46 (60)	29 (38)	(?)
Nickel workers	318	181 (57)	123 (39)	14 (4)
Controls	57	42 (74)	14 (24)	1 (2)
All	375	223 (59)	137 (37)	15 (4)

Includes 2 men with nasal carcinoma, diagnosed by rhinoscopy as nasal polyps
One man 32 years old with allergic rhinitis.

Statistical analysis

In an attempt to explain the rhinoscopical findings (Y) from 0 to 3 by means of several independent variables (X_1 X_2 X_7) the following model equation was applied

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7$$

where

a = constant factor
b_1 b_2 b_7 = regression coefficients
X_1 = 1 if the subject was working with crushing roasting and smelting otherwise 0
X_2 = 1 if the subject was working with electrolytic processes otherwise 0
X_3 = 1 if the subject was working with non-process work at the refinery otherwise 0
X_4 = the age of the subject in years
X_5 = number of years from first employment at the plant
X_6 = grams tobacco per week
X_7 = μg Ni/100 g nasal mucosa wet weight

To test the explanatory values of each of the independent variables a stepwise multivariate regression analysis forward method was performed by calculating simple and partial correlation coefficients between the rhinoscopical findings (Y) and each of the independent variables (X_1 X_2 X_7) This analysis was based on a standard program for multiple regression (NRSR) developed at The Norwegian Computing Centre Blindern Oslo and conducted on a Univac 1108 computer The *final equation* was calculated from the above-mentioned model The significance levels for the correlation coefficients were tested with Student s *t* test The χ^2 test was applied for calculating the significance levels of some tabulated differences The significance levels for differences between means were calculated with Student s *t*-est A level of statistical significance of less than 5% was required

RESULTS

Subjective nasal complaints and rhinoscopical findings

The subjective nasal complaints in nickel workers and in controls are summarized in Table II No predominant nasal symptom was found in the nickel-exposed group as compared with the controls

Forty two of the controls (74%) and 181 of the nickel workers (57%) showed normal

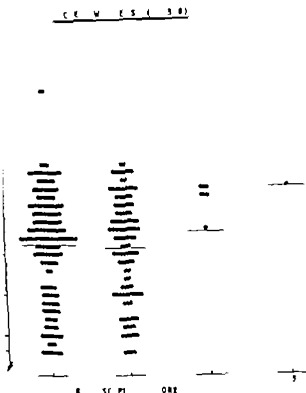


Fig. 1. Rhinoscopical scores (0–3) and duration of nickel exposure in 318 nickel workers. The mean exposure time for each rhinoscopical group is marked with horizontal lines.

inoscopical findings (Table III). The increased number of cases with pathological findings in the nickel-exposed group (43%) compared with the control group (26%) is statistically significant (0.01<P<0.02). No significant difference of the rhinoscopical findings was observed between the three work categories in the nickel-exposed group.

Hyperplastic rhinitis was the most common pathological finding, both in controls and in nickel workers, and was also mainly responsible for the difference in frequency of pathological findings between the nickel-exposed group and the controls. Moreover, the mucosal hyperplasia in the nickel workers seemed to be more pronounced and localized to the middle turbinate as compared with the controls, giving the surface an uneven appearance with small point like impressions. Polypoid mucosa with or without polyps was diagnosed in 13 nickel workers (4%) (Fig. 1) and in one of the controls (2%) (Table III). Again, the nickel workers showed more pronounced changes, often with single or multiple polyps. Nasal polyps in 2 subjects working with roasting and smelting processes appeared at histological examination to be nasal carcinomas. These workers were in full employment without serious symptoms at diagnosis. Both had started at the nickel refinery 28 years previously. One nickel worker was clinically suspected to have a neoplastic tumorous lesion of the middle turbinate, but the diagnosis was not confirmed by histological examination. In the nickel-exposed group 3 cases of perforation of the nasal septum were found, in 2 probably following an operative resection of the nasal septum. No cases of pronounced mucosal athrophy were observed.

Rhinoscopy and duration of nickel exposure

Figure 1 shows the association between the rhinoscopical scores and the duration of nickel exposure in the 318 nickel workers. The *sim-*

Table III *Rhinoscopical findings in nickel workers distributed by category of work, and in controls*

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Controls	57	4? (74)	14 (24)	1[b] (2)
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Includes ? men with nasal carcinoma diagnosed by rhinoscopy as nasal polyps
One man, 3? years old with allergic rhinitis

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where

a = constant factor

b_1 b_2 b_7 = regression coefficients

X_1 = 1 if the subject was working with crushing roasting and smelting otherwise 0

X_2 = 1 if the subject was working with electrolytic processes otherwise 0

X_3 = 1 if the subject was working with non-process work at the refinery otherwise 0

X_4 = the age of the subject in years

X_5 = number of years from first employment at the plant

X_6 = grams tobacco per week

X_7 = µg Ni/100 g nasal mucosa wet weight

To test the explanatory values of each of the independent variables a stepwise multivariate regression analysis forward method was performed by calculating simple and partial correlation coefficients between the rhinoscopical findings (Y) and each of the independent variables (X_1 X_2 X_7) This analysis was based on a standard program for multiple regression (NRSR) developed at The Norwegian Computing Centre Blindern Oslo and conducted on a Univac 1108 computer The *final equation* was calculated from the above mentioned model The significance levels for the correlation coefficients were tested with Student t test The χ^2-test was applied for calculating the significance levels of some tabulated differences The significance levels for differences between means were calculated with Student s t-test A level of statistical significance of less than 5% was required

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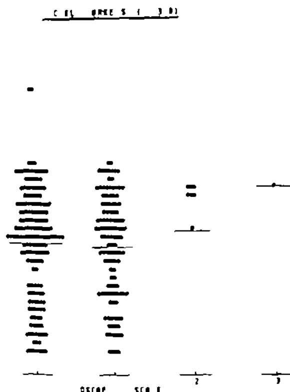


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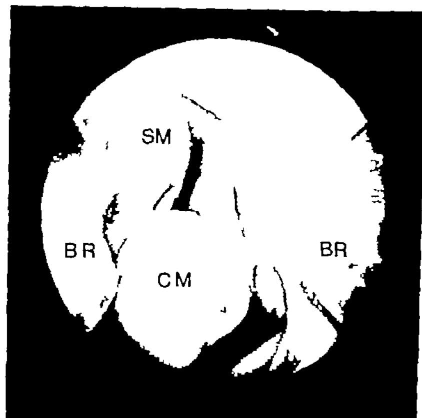


Fig ? Hyperplastic nasal middle turb after application of adrenalin to the m membrane The photo refers to a 57- old electrolytic worker employed a nickel refinery for 78 years and smoki grams tobacco per week. Photogra through Wolf Lumina, 170° *CM* Co media *SM* septal mucous membrane branches of the nasal speculum.

ple correlation between these two parameters in not statistically significant ($r=0.096$, $n=375$, $P<0.06$) whereas the *partial* correlation is statistically significant ($r=0.100$, $n=375$, $P<0.05$) (Table VIII)

Rhinoscopy smoking habits and tobacco consumption

The frequency of subjects who smoked was about the same in both groups in the material (Table IV) The nickel workers howe smoked larger amounts of tobacco than controls This difference in tobacco consu tion is not statistically significant There tendency towards more smokers in gro with abnormal rhinoscopy Both *simple* *partial* correlations between the rhinoscop scores (Y) and the tobacco consumptio grams per week (X_4) are statistically sig cant ($P<0.05$) (Table VIII)

Table IV *Tobacco smoking in nickel workers and in controls distributed by rhinoscopical fi ings*

	Nickel workers				Controls			
Rhinoscopical findings	No of subject with clinical findings *n*	Non-smoker (%)	Smokers *n* (%)	Grams tobacco/ smoker/ week	No of subjects with clinical findings *n*	Non-smokers *n* (%)	Smokers *n* (%)	Gram tobac smok week
No abnormalities	181	75 (41)	106 (59)	77 5	4	18 (43)	4 (57)	74 4
Hyperplastic rhinitis	1_3	41 (33)	87 (67)	84 6	14	3 (1)	11 (79)	70.3
Polypoid mucosa polyps/tumou	14	5 (36)	9 (64)	81 7	1	0 (0)	1 (100)	75 0
Total	318	1 1 (38)	197 (6_)	80 7	57	1 (37)	36 (63)	73_

able V *The mean nickel content in hand-rolled cigarettes of Norwegian prepared tobacco nd in one type of U.S. produced cigarette*

igarette types	Mean nickel content ± 1 S.D. (range) in		Mean nickel content/cigarette (µg/cigarette)
	Tobacco (µg/g fresh wt.)	Cigarette paper (µg/paper)	
[illegible] tobacco (Tiedemanns gul) ($N=10$)	*0.29*±0.10 (0.1–0.4)	*0.05*±0.03 (0.0–0.1)	*0.34*
U.S. cigarettes (Pall Mall) ($N=10$)	*2.08*±0.65 (0.8–3.2)	*0.05*±0.03 (0.0–0.1)	*2.13*
Cigarettes rolled by 10 electrolytic work. (Tiedemann) ($N=10$)	*4.81*±2.98 (1.7–10.6)	*6.53*±6.20 (0.7–22.6)	*11.34*
Cigarettes rolled by 10 roast/smelt. ork. (Tiedemann) ($N=10$)	*13.34*±8.59 (1.1–29.7)	*27.63*±40.6 (2.[illegible]–128.0)	*40.97*

The nickel content in tobacco and cigarette papers is shown in Table V. The tested U.S. tobacco contained about 7 times more nickel than the Norwegian. The cigarettes rolled by the nickel process workers were markedly nickel-contaminated as compared with the cigarette material. The cigarette papers in particular were heavily contaminated. The cigarettes rolled by the subjects working with roasting and smelting processes were particularly nickel enriched with an averagely increased content about 120 times the non-contaminated level. The correlation between the nickel content in paper and tobacco of the 20 nickel-contaminated cigarettes is statistically significant ($r=0.72$, $P<0.01$).

Rhinoscopy and histopathology

Table VI and VIII demonstrates the lack of correlation between rhinoscopical findings and histology of the mucosal biopsies in nickel workers and controls.

X-ray examination

No significant difference in sinus X-ray findings was found between nickel workers and controls (Table VII). Even in subjects with markedly pathological rhinoscopy the X ray examination revealed normal findings in more than 50% of the cases. One of the men with a nasal carcinoma showed normal X ray findings. In the second case X-ray revealed mark

Table VI *Rhinoscopical findings in nickel workers and in controls distributed by histological findings*

Histological changes of the nasal mucosa	Nickel workers			Controls		
	No. of subjects with histological findings	Normal rhinoscopy (%)	Abnormal rhinoscopy (%)	No. of subjects with histological findings	Normal rhinoscopy (%)	Abnormal rhinoscopy (%)
Without carcinoma/epithelial dysplasia	278	159 (57)	119 (43)	56	42 (75)	14 (25)
With carcinoma/epithelial dysplasia	40	22 (55)	18 (4[illegible])	1	0 (0)	1 (100)
Total	318	181 (57)	137 (43)	57	42 (74)	15 (26)

Includes men with nasal carcinoma.
A carpenter 65 years old with epithelial dysplasia.

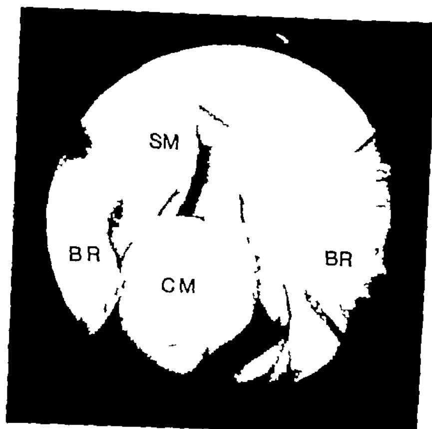


Fig 2 Hyperplastic nasal middle turbinate after application of adrenalin to the mucous membrane The photo refers to a [illegible] year old electrolytic worker employed at the nickel refinery for [illegible] years and smoking [illegible] grams tobacco per week Photographed through Wolf Lumina 170° CM Concha media SM septal mucous membrane BR branches of the nasal speculum

ple correlation between these two parameters is not statistically significant ($r=0.096$ $n=375$ $P<0.06$) whereas the *partial* correlation is statistically significant ($r=0.100$ $n=375$ $P<0.05$) (Table VIII)

Rhinoscopy smoking habits and tobacco consumption

The frequency of subjects who smoked was about the same in both groups in the material (Table IV) The nickel workers however smoked larger amounts of tobacco than the controls This difference in tobacco consumption is not statistically significant There is a tendency towards more smokers in groups with abnormal rhinoscopy Both *simple* and *partial* correlations between the rhinoscopical scores (Y) and the tobacco consumption in grams per week (X) are statistically significant ($P<0.05$) (Table VIII)

Table IV *Tobacco smoking in nickel workers and in controls distributed by rhinoscopical findings*

Rhinoscopical findings	Nickel workers: No of subjects with clinical findings	Non-smokers n (%)	Smokers n (%)	Grams tobacco/smoker/week	Controls: No of subject with clinical findings n	Non-smokers n (%)	Smokers n (%)	Grams tobacco/smoker/week
No abnormalities	181	75 (41)	106 (59)	77 5	4	18 (43)	4 (57)	74 4
Hyperplastic rhinitis	1_3	41 (33)	8? (67)	84 6	14	3 (1)	11 (79)	70 3
Polypoid mucosa polyps/tumour	14	5 (36)	9 (64)	81 7	1	0 (0)	1 (100)	75 0
Total	318	1 1 (38)	197 (6_)	80 7	57	1 (37)	36 (63)	73_

scopical findings were chosen on the basis of previous experiences from the above-mentioned pilot study

The rhinoscopical findings revealed few specific characteristics of the nasal mucosa in nickel workers even though the number of abnormal findings was increased and, on ... they were more pronounced than in the controls The lack of association between rhinoscopy and histology limits the value of these findings however Two cases among 13 with a rhinoscopical diagnosis of nasal polyps (15%) turned out to be nasal carcinomas, by histological examination This leads us to conclude that all nickel workers with nasal polyps should be subjected to biopsy and histological examination

Reports comparable to ours are scarce Tatarskaya (1960) and Kucharin (1970) reported increased frequency of chronic atrophic rhinitis sinusitis and severe damage to the nasal mucosa in workers who were chronically exposed to inhalation of nickel aerosols Their results which are not consistent with our findings were based on incompletely described diagnoses and without the use of controls.

The frequencies of the abnormal radiograms were fairly equal in nickel workers and in controls and did not correlate with the rhinoscopical findings Moreover the radiological examination revealed few characteristic findings One possible explanation is that the early mucosal changes in nickel-induced carcinoma start in the nasal cavity adjacent to the middle turbinate and first at a later stage affect the paranasal sinuses (Virtue 197? Torjussen et al 1978)

The multiple regression analysis indicated the number of years from first employment at the nickel refinery and the amounts of tobacco consumed as the only factors that partly could explain the rhinoscopical findings and rejected the explanatory value of work categories age and nickel content of the nasal mucosa. It is interesting to state that tobacco among several other carcinogens also contains nickel Sunderman et al (1975) summarized reports on the mean nickel content of various sources of tobacco from 2 µg to 6 2 µg/cigarette consistent with our results. These figures are far below the presently reported amounts of nickel in cigarettes hand-rolled by nickel workers It remains to be analysed how much nickel is contained in such contaminated cigarettes and which can be retrieved from the cigarette smoke that when inhaled leads to increased exposure of the respiratory epithelium to nickel compounds

The conclusion to be drawn from this study is that rhinoscopy and X-ray examination are inadequate screening methods for detecting early carcinoma or precancerous mucosal changes in nickel workers Although the frequency and the degree of pathological findings by rhinoscopy are higher in nickel-exposed individuals the clinical examination must be combined with histological or possibly cytological examination in order to determine the nature of the mucosal changes

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Rhinoskopie und Röntgenuntersuchungen wurden an 318 Nickelarbeitern und 57 Kontrollen durchgeführt, um die Bedeutung dieser Methoden bei der Entdeckung von präkanzerösen und kanzerösen Veränderungen der Schleimhaut zu studieren. Die klinischen und radiologischen Untersuchungen wurden verglichen mit histopathologischen Befunden und Nickelkonzentrationsmessungen der Schleimhaut der mittleren Nasenmuschel und in Beziehung gesetzt zur Nickelexponierung und den Rauchgewohnheiten. Der Nickelgehalt der Zigaretten wurde bestimmt. Die nickelexponierten Personen hatten statistisch signifikant (0,01<P<0,02) mehr pathologische Veränderungen (43%) als die Kontrollen (26%), hauptsächlich als Folge der Unterschiede in der Häufigkeit hyperplastischer Rhinitis. 13 Nickelarbeiter (4%) hatten Nasenpolypen. In ... dieser Fälle die 28 Jahre lang in einer Nickelraffinerie gearbeitet hatten, zeigte die histologische Untersuchung ein Nasenkarzinom. Keine eindeutige Beziehung wurde gefunden zwischen den rhinoskopischen Befunden und histologisch erkannten Epi-

Table VII *Sinus X ray findings in nickel workers and in controls distributed by rhinoscopic findings*

Rhinoscopical findings	Nickel workers: No of subjects with clinical findings n	Nickel workers: Normal X-ray findings n (%)	Nickel workers: Abnormal X-ray findings n (%)	Controls: No of subjects with clinical findings n	Controls: Normal X-ray findings (%)	Controls: Abnormal X-ray findings (%)
No abnormalities	181	164 (91)	17 (9)	42	37 (88)	5 (12)
Hyperplastic rhinitis	123	106 (86)	17 (14)	14	10 (71)	4 (29)
Polypoid mucosa polyps/tumour	14	8 (57)	6 (43)	1	1 (100)	0 (0)
Total	318	278 (87)	40 (13)	57	48 (84)	9 (16)

edly pathological changes compatible with bony destruction

Statistical analysis and final equation

Table VIII shows the *simple* and *partial* coefficients of correlation between the rhinoscopical scores (Y) and the different explanatory factors (X_1 X_2 X_7) applied in the model equation Only tobacco consumption (X_6) and number of years from first employment at the nickel refinery (X_5) showed a statistically significant correlation to the scores for the rhinoscopical findings whereas no correlations were found between rhinoscopical scores and working categories (X_1 X_2 X_3) age (X_4) and nickel content in nasal mucosa (X_7) (Table VIII)

Hence the calculation gave the following final equation

$$Y = 0\ 279 + 0\ 00584 X_5 + 0\ 00131 X_6$$

Table VIII *Simple and partial correlation coefficients between rhinoscopical scores (Y) from 0 to 3 and some explanatory factors* (see text)

Correlation between rhinoscopical score (Y) and	Correlation coefficients ($N = 375$): Simple	Partial
Roasting/smelting (X)	0.08453	–
Electrolysis (X)	0 03340	–
Non-process work (X_3)	–0 02319	–
Age in years (X)	0.04909	–
Years from first nickel exposure (X_5)	0 09645	0 1000
Grams tobacco per week (X_6)	0.10669*	0 10997
µg Ni/100 g nasal mucosa (X)	0.08365	–

$P < 0\ 05$

DISCUSSION

The present clinical study is part of a comprehensive survey on the carcinogenic risk in nickel workers where histological chemical and histochemical techniques have been applied on different types of samples collected from the same subjects (Torjussen & Haug 1978 Torjussen & Solberg 1978 Torjussen et al 1978 Torjussen & Solberg 1978 in press) A histopathological pilot study of nasal biopsies revealed carcinoma and epithelial dysplasia in nickel workers with at least 10 years employment in process work (Torjussen & Solberg 1976 1978) Planning of the present material was based upon these findings. The hospital patients used as controls were chosen as being convenient subjects for contributing nasal biopsies

The rhinoscopical examinations and the judgement of the findings were based on the examiner s previous training and experience Precautions against the influence of bias were taken as far as practically possible The applied characteristics for evaluating the rhino-

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CARCINOMA OCCURRING IN BRANCHIAL CLEFT CYSTS

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(Received October 3 1978)

Abstract In order to find histological data in the differentiation between branchial cleft carcinomas and metastatic carcinomas the specimens from 154 patients with branchial cleft cysts and 7 patients with an isolated tumour in the neck with unknown primary tumour were reviewed and compared with 10 normal lymph nodes. Absence of lymph node structures as peripheral lobulation, internodular trabeculae and perinodular sinuses in branchial cleft cysts, are found valuable for distinguishing primary carcinoma of branchial cleft cyst from metastases. A correct diagnosis of this rare tumour is important in order to avoid overtreatment of these patients, who have a good prognosis if treated with surgical excision only

Branchial cleft cysts are congenital anomalies located along the anterior border of the sternocleidomastoid muscle and occur most frequently in the upper one third of the neck (Dehner 1975) Microscopically the cysts are lined by different types of epithelium The cyst wall contains varying amount of lymphoid tissue *with or without germinal centres*

The fact that the branchial cleft cysts are located in the same region as the deep cervical lymph nodes makes it difficult or impossible to distinguish a primary carcinoma occurring in the epithelium of a branchial cleft cyst from a metastatic lesion

As the treatment and the prognosis of branchial cleft carcinoma (BCC) and metastatic carcinoma are essentially different and make neoplastic diseases in this region a differential diagnostical problem a review of BCC which are diagnosed in a 10-year period at the department of pathology Rigshospitalet was performed

MATERIAL AND METHODS

The material was obtained from consecutive operations carried out in the period 1967–76 at the department of maxillofacial surgery and the department of otolaryngology Rigshospitalet Copenhagen 161 patients were operated on for a mass in the lateral aspect of the neck with no other primary tumours elsewhere

The diagnosis of branchial cleft cyst was made in 154 patients 68 of these patients were men ages at operation ranging from 19 to 76 years (average 41) and 70 were women ages ranging from 16 to 73 years (average 33) There were 16 children, ages ranging from 2 to 14 years (average 8) There were 9 boys and 7 girls

Carcinoma was the diagnosis in 7 patients 6 of these were men ages at operation ranging from 53 to 76 years (average 62)

The paraffin sections from all branchial cleft cysts and carcinoma were reviewed histologically The presence of normal lymph node structures was registered The specimens from the carcinomas were re-cut and stained with *haematoxylin-eosin* Van Gieson–Hansen s stain periodic acid Schiff (PAS) and silver impregnation

RESULTS

Benign lesion

When reviewing the 154 branchial cleft cysts a comparison with 10 normal lymph nodes from the same region was performed.

theldysplasien Der Erkenntniswert für die rhinoskopischen Befunde von solch verschiedenen Faktoren wie Arbeitskategorie Alter Exponierungsdauer Tabakverbrauch in g/Woche Nickelgehalt der Nasenschleimhaut wurde durch eine stufenweise Multivarianz Regressions-Analyse evaluiert. Nur die Beschäftigungsdauer in der Nickelraffinerie und der Tabakverbrauch zeigten eine statistisch signifikante Korrelation zu den rhinoskopischen Befunden Die Röntgenuntersuchungen zeigten wenig charakteristische Befunde Die chemische Untersuchung der durch die Nickelarbeiter selbstgerollten Zigaretten zeigte einen hohen Nickelgehalt verglichen mit nichtkontaminierten Zigaretten

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Table I *Histological findings in 154 branchial cleft cysts*

Structures occurring in branchial cleft cysts	Numbers	Per cent
Normal epithelium	154	100
Thick connective tissue capsule with lymphocytes	27	18
Subcapsular sinus	80	52
Medullary sinuses	42	27
Germinal centres	94	61
Thin connective tissue capsule	127	82

The morphology in the lymphatic tissue in lymph nodes was found to differ clearly from the lymphatic tissue in the wall of branchial cleft cysts (Figs 1 and 2) No peripheral lobulation characteristic for the cortical area in lymph nodes was seen in the cysts Neither internodular trabeculae nor perinodular sinuses were seen in any of the cysts

Subcapsular sinus medullary sinus germinal centres and a thin connective tissue capsule were common features and were seen in 52% 27% 61% and 82% respectively of the branchial cleft cysts (Table I) Normal epithelium and a thick connective tissue capsule with varying numbers of lymphocytes were seen only in the cysts Normal epithelium was seen in all the cysts A thick connective tissue capsule was found in 18% of the cases

Malignant lesion

When reviewing the seven carcinomas found in patients with an isolated mass in the neck and no primary tumour elsewhere the morphologic differences found in the lymphatic tissue of the branchial cleft cysts and the lymph nodes were used (Table II) Two cases were excluded according to these criteria as being metastatic carcinomas

As seen in Table II one case (case III) only shows structures occurring in both BCC and lymph nodes and could not be classified by histological preparations alone but an aspiration biopsy performed one month before operation showed normal ciliated columnar epithelium cells This information confirmed the diagnosis as normal ciliated columnar epithelium cells cannot be found in a metastasis from a squamous cell carcinoma In 4 cases a carcinoma in a wall typical of a branchial cleft cyst, lined by an atypical epithelium could be demonstrated (Figs 3 and 4)

The most important clinical data in the 5 patients with BCC are presented in Table III

An extensive search for another primary tumour in all patients was negative

Only one patient (case II) had been treated for a malignant tumour—3 years earlier It was a well-differentiated squamous cell carcinoma on the right ear There was no sign of recurrence when he got a carcinoma in the branchial cleft cyst which clinically had been present for 30 years

None of the patients had metastases at operation. In case IV two tumours were found but histologically both were branchial cleft cysts with carcinoma arising in the wall.

Table II *The histologic featu es of the cyst wall in five cases of diagnosed branchial cleft carcinoma*

Structures occurring in branchial cleft cysts	Case I	Case II	Case III	Case IV		Case V
				Tumour 1	Tumour 2	
Normal and atypical epithelium	+	+	−	+	+	+
Thick connectiv tissue capsule	+		−	−	+	−
Subcapsular sinus	−	−	+	−	+	+
Medullary sinuses	−	−	+		−	+
Germinal centres	−	−	+	+	−	+
Thin connectiv tissue capsule	−	−	+		−	+



Fig 1 Lymph node showing the characteristic lobulated *cortical* area. The germinal centres are surrounded by internodular trabeculae and perinodular sinuses [illegible] by arrows (HE, ×35)



Fig 2 Branchial cleft cyst lined by squamous epithelium with abundant lymphoid tissue and germinal centres in the connective tissue wall No peripheral lobulation Internodular trabeculae or perinodular sinuses are seen (HE, ×35) (Compare Fig. 1)

Table III *Clinical data in five cases of carcinoma in branchial cleft cysts found in a 10-year period from 1967 to 1976*

Case no	Case I	Case II	Case III	Case IV	Case V
Age at operation/sex	70/M	76/M	53/F	68/M	57/M
Duration before operation	months	30 years	6 weeks	6 months	12 months
Location in carotid triangle	Right	Right	Left	Left	Left
Finding at operation	Cyst	Cyst	Cyst	Necrotic metastases	Cyst
Treatment in addition to excision	Neck diss		Radiation therapy	Neck diss, radiation therapy	Radiation therapy
Observation time (years) without recurrence or other primary tumours	10 alive	8 alive	5 alive	3 alive	11 alive
Histological diagnosis	Branchial cleft carcinoma	Cystic and solid carcinoma	Branchial carcinoma or metastasis	Possible branchial cleft carcinoma	Possible branchial cleft carcinoma
Histological diagnosis by reviewing	BCC	BCC	BCC	BCC	BCC

The indication for postoperative radiation therapy was the uncertainty of the diagnosis. In one patient (case II) there was a rapidly growing local recurrence after the out-patient removal of the cystic tumour in the neck.

DISCUSSION

The existence of BCC has been disputed and questioned since Martín, Morfit & Ehrlich (1950) reviewed 250 cases published in the world literature and stated that they had not seen a single proven case. For the hypothetical diagnosis of BCC Martin and his co-workers established four criteria.

(1) The cervical tumour must have occurred somewhere along a line extending from a point just anterior to the tragus of the ear downward along the anterior border of the sternomastoid muscle to the clavicle.

( ) The histological appearance of the growth must be consistent with an origin from tissue known to be present in branchial vestiges.

(3) The patient must have survived and have been followed by periodic examinations for at least 5 years without the development of any other lesion which could possibly have been the primary tumour.

(4) The best criterion of all would be the histologic demonstration of a cancer developing in the wall of an epithelial-lined cyst situated in the lateral aspect of the neck.

Since 1950 another 15 cases have been published. Only one case described by Benisch & Som (1973) satisfies all four criteria established by Martin et al. None of the other cases fulfils the third criterion (Bernstein, Scardino, Tomaszewski & Cohen 1976, Collins & Edgerton 1959, Goldschmidt 1961, Hansson & Lindström 1972, Katubig & Damjanov 1969, Rossberg & Rosemann 1964, Schuring & Arthur 1967, Stackpole & Pearce 1961, Stockdale 1960, Strong & Sommers 1958) but all meet the fourth criterion which Martin and his co-workers themselves regard as being the only absolute proof for the diagnosis of BCC. Two cases described by Lane (1958) lack picture documentation.

In the present material all 5 cases satisfy the first and the second criterion. Cases I and III satisfy the third and all but case III satisfy the fourth criterion.

By reviewing those cases reported after 1950 including the present series only 2 cases out of 18 cases satisfy all the four criteria (Benisch et al., present series case I). 15 cases out of 18 cases fulfil the fourth criterion. In



Fig 3 Branchial cleft cyst lined by squamous epithelium with epithelial atypia. The cyst wall is infiltrated by carcinoma of low differentiation (*arrow*) (case II HE ×140)



Fig 4 Segment of branchial cleft cyst with papillary squamous cell carcinoma. The cyst wall shows abundant lymphoid tissue with medullary sinuses No peripheral lobulation internodular trabeculae and perinodular sinuses are seen (case V HE, ×35)

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view of these well-documentated cases I believe that there should be a modification of these criteria.

The location of BCC makes it possible to be mistaken for metastatic carcinoma Histologically the diagnosis of BCC can be made only when a cyst wall clearly exists without perinodular sinuses internodular trabeculae and peripheral lobulation Metastatic cancer must be suspected in all cases where doubt exists

Isolated tumours in the neck where no primary lesion is found do not preclude a metastatic cancer as cervical metastases with a silent primary lesion occur in about 16% of all cases of cancer in the nasopharynx (Hendrick 1967)

A 5-year survival is an important supplementary information in the retrospective work but has only theoretical importance as the diagnosis of BCC has to be made in the actual situation so that the therapy can be prepared accordingly

Another primary tumour (Strong et al case II) developed within five years does not exclude a BCC at the same time It may be a manifestation of the well-known phenomenon that two tumours in the upper respiratory tract can develop simultaneously or shortly after each other

Bernstein et al exclude all cases of well-documentated BCC in patients who have received irradiation towards the neck because a possible primary tumour may be fallen within the beam of cancer lethal radiation which does not exclude a BCC

The treatment of tumours of the neck depends on the histological diagnosis Metastases from a primary unknown tumour are treated with megavolt radiation while BCC are treated with excision of tumour and perhaps neck dissection In the present material 4 out of 5 patients with BCC have received post operative radiation therapy the indication in 3 of the cases was the uncertainty of the diagnosis

To diagnose BCC the whole cyst must be cut up so that the material includes many specimens The most important is to search for remains of normal epithelium

Germinal centres medullary sinuses and subcapsular sinus are structures normally occurring in a branchial cleft cyst On the other hand peripheral lobulation internodular trabeculae or perinodular sinuses are not found, why the occurrence of these structures excludes the diagnosis of BCC if a cyst wall with normal epithelium does not exist.

Cases where an atypical carcinomatous epithelium lines the cysts comprised for instance of necroses or keratin accumulation must not be mistaken for BCC

Even if BCC is a rare tumour the diagnosis is important in order to avoid over treatment of these patients who have a good prognosis compared with patients with metastatic cancer So one cannot wait to establish the diagnosis until all four criteria are fulfilled but must work towards the diagnosis BCC as a pathologic entity

ZUSAMMENFASSUNG

Um histologische Kriterien zur Unterscheidung von branchiogenen Carcinomen und Carcinommetastasen zu finden wurden histologische Proben von 154 Patienten mit branchiogenen Cysten und von 7 Patienten mit einem solitären Halstumor ohne erkannten Primärtumor untersucht und mit 10 normalen Lymphdrüsen verglichen. Auf Grund fehlender Lymphdrüsenmerkmale wie periphere Lobulierung internoduläre Trabeculae und perinoduläre Sinusoide in branchiogenen Cysten, ist es dem Autor möglich ein primäres branchiogenes Carcinom von Metastasen zu unterscheiden Die präzise Diagnose dieses seltenen Tumors ist wichtig, um eine Überbehandlung dieser Patienten zu verhindern die nach chirurgischer Excision allein eine gute Prognose haben.

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INHERITED CONGENITAL BILATERAL ATRESIA OF THE EXTERNAL AUDITORY CANAL CONGENITAL BILATERAL VERTICAL TALUS AND INCREASED INTEROCULAR DISTANCE

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(Recei ed November 4 1978)

Abstract Six out of twenty descendants of a reportedly affected grandfather have congenital bilateral symmetrical and isolated subtotal atresia of the external auditory canal Four of the six affected descendants have bilateral foot anomalies—two affected cousins having congenital vertical talus All of the three affected boys in the third generation have increased interocular distance Short fifth fingers, bilateral single transverse palmar creases pyloric stenosis and congenital exotropia were found infrequently and are considered coincidental features Apart from the atresia, oto-rhinolaryngologic examination mental function dermatoglyphics IgA kidney function and heart function of the affected descendants were all normal The karyotype of four affected descendants examined was normal An autosomal dominant inheritance with variable expressivity is suggested

The association of congenital first and second pharyngeal arch malformations with congenital anomalies of the extremities is frequently described in the literature Some of the cases fit well-defined syndromes (Konigsmark & Gorlin 1976) whereas others seem to occur sporadically (Rapin & Ruben 1976)

The present syndrome—which we could not find described in the earlier literature—reveals an inherited association between congenital bilateral symmetrical and isolated sub-total atresia of the external auditory canal congenital bilateral foot deformities—especially congenital vertical talus—and an increased interocular distance

MATERIAL AND METHODS

The interocular distance is described by the formula

$$IP = 0\ 17 + 0\ 59 \times IC + 0\ 41 \times OC$$

(corrected formula, original formula erroneous) where IP = interpupillary IC = inner canthal and OC = outer canthal distance (Feingold & Bossert 1974)

By using an IP/age graph with percentiles, an estimate of possible ocular hypertelorism is obtained

The interocular distance is also expressed by the canthal index = (IC/OC) × 100 (Günther 1933) Both values are given in Table I

Dermatoglyphics (shown in Table II) are designated according to Holt (1968) and Penrose & Loesch (1970)

Karyotyping was performed using the G-banding technique as well as the reverse-banding technique (Dutrillaux & Lejeune 1971)

The family members will be described according to the numbering in the pedigree (Fig 1)

I 1 The grandfather died several years ago He was hard-of hearing and had something with his feet Further information is not available He had been married once earlier the

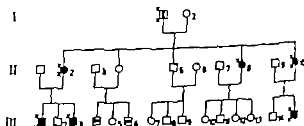


Fig 1 Pedigree Symbols ● ■ = examined affected ◫ = reportedly affected = congenital bilateral atresia, = congenital bilateral foot-deformities & mental deficiency

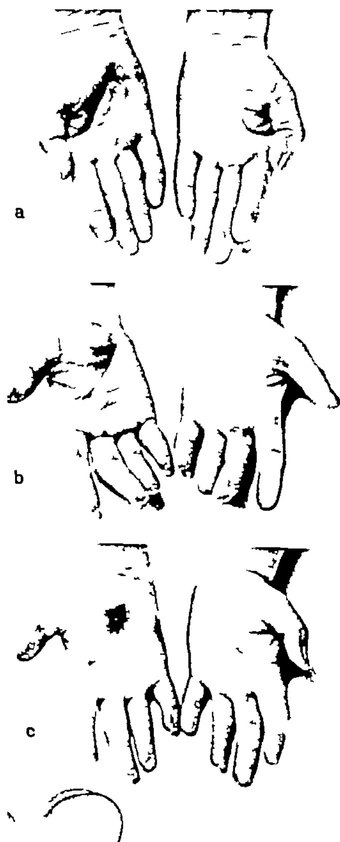


Fig. 2. Hands of three family members: ( ) II.1 (b) III.1 ( ) III.3

Table I *Interocular distance of three examined affected family members*

	Age (years)	IC	OC	IP	Percentile	CI
III 1	14	3 7	9.2	6 13	75%	40.2
III 3	10	3 3	9 1	5 85	80%	36 3
III 15	8	3.3	8 3	5.5	75%	39.8

Normal<38<euryopia<4 <hypertelorism

present family having no contact with the offspring of this earlier marriage It has not been possible to trace these descendants

I 2 Reportedly normal She too died several years ago and no further information is available

II 1 The reason for the examination of this non-descendant was a peculiarity of the fifth finger on both hands bearing some resemblance to the fifth fingers of his two affected sons III 1 and III 3 (Fig 2) Apart from this bilateral clinodactyly normal dermatoglyphics were found (Table II)

II 2 A 47 year-old female with congenital bilateral atresia of the external auditory canal and congenital bilateral club foot Examination of the hands revealed slight bilateral clinodactyly with normal dermatoglyphics. Normal appearance and position of the pinnae. Normal position of the external auditory canal No cervical cysts or fistulas No preauricular pits No increased interocular distance left normal No heart or kidney defects No mental retardation Present hearingloss TI right 55 dB TI left 45 dB Normal bone conduction She has never been treated for atresia or club foot Normal karyotype

II 4 Reportedly normal

II.5 Reportedly normal

II 8 A 42 year-old female with congenital bilateral atresia of the external auditory canal No other deformities are recorded No mental retardation The right ear was operated on in 1951 and the left ear in 1954 both at other clinics From the operation in 1954 it is recorded that the ossicles were normal although the mobility of the malleus was decreased. A reoperation of the left ear was performed at our clinic in 1966 No further information can be gathered from this operation because of the earlier removal of ossicles and severe inflammation Vestibular function was assessed by rotation and found to be normal Hearing-loss

Table II *Dermatoglyphics of three examined family members*

w = whorls u = ulnar loops r = radial loops
atd = maximal atd angle

	Fingers										
	Left					Right					
	V	IV	III	II	I	I	II	III	IV	V	TRC
II 1	w	w	u	r	w	w	w	w	w	w	183
III 1	u	w	u	r	u		r	u	w	u	122
III 3	u	w	w	w	w	w	w	w	w	u	164

	Palms						
	Left			Right			a–b count
	Loops	Triradii	atd	Loops	Triradii	atd	
II 1	IV V	tt"4	60°	I III V	tt"e4	73	77
III 1	III	t4	35°	III	t4	37°	69
III 3	III V	tt 4	68°	III V	tt 4	36	77



Fig. 3. Increased interocular distance and left exotropia in III.1

in 1966: TI right 60 dB TI left, 55 dB. Normal bone conduction. She has not been seen at our clinic since 1966.

II 10. A 39-year-old female with congenital bilateral symmetrical narrowing of the external auditory canal and congenital bilateral pes excavatus. No increased interocular distance. Normal appearance and position of the pinnae. Normal position of the external auditory canal. No cervical fistulas or cysts. No preauricular pits. The ear drums could not be visualized. Impedance audiometry revealed normal middle ear pressures, small middle ear compliances (0..5 cc) bilaterally, normal stapedial reflexes in the right ear but absent reflexes in the left. Present hearing loss: TI right 70 dB, TI left 70 dB. Normal bone conduction.

III 1. A 14-year-old boy with congenital bilateral atresia of the external auditory canal, congenital pes excavatus dxt, congenital talus verticalis sin, strabismus divergens sin, increased interocular distance (Fig. 3 and Table I) and bilateral clinodactyly (Fig. b). Normal



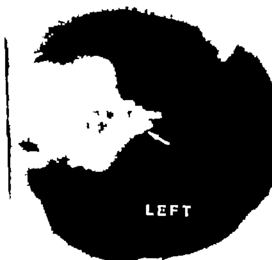


Fig. 4. Tomography of the temporal bones in III 1. (Arrows indicate bony atresia plate.)

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III 1	14	3 7	9 2	6 13	75%	40..
III.3	10	3 3	9 1	5 85	80%	36.3
III 15	8	3 3	8 3	5.5	75%	39 8

Normal<38<euryopia<4 <hypertelorism

present family having no contact with the offspring of this earlier marriage It has not been possible to trace these descendants

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II 4 Reportedly normal

II.5 Reportedly normal

II 8 A 42 year-old female with congenital bilateral atresia of the external auditory canal. No other deformities are recorded No mental retardation The right ear was operated on in 1951 and the left ear in 1954 both at other clinics From the operation in 1954 it is recorded that the ossicles were normal although the mobility of the malleus was decreased A reoperation of the left ear was performed at our clinic in 1966 No further information can be gathered from this operation because of the earlier removal of ossicles and severe inflammation Vestibular function was assessed by rotation and found to be normal Hearing-loss

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w = whorls u = ulnar loops r = radial loops
atd = maximal atd angle

	Fingers										
	Left					Right					TRC
	V	IV	III	II	I	I	II	III	IV	V	
II 1	w	w	u	r	w	w	w	w	w	w	183
III 1	u	w	u		u	u		u	w	u	1..
III 3	u	w	w	w	w	w	w	w	w	u	164

	Palms						
	Left			Right			a–b count
	Loops	Triradii	atd	Loops	Tririad	atd	
II 1	IV V	tt'4	60°	I III V	tt'e4	73°	77
III 1	III	t4	35°	III	t4	37°	69
III.3	III V	tt 4	68°	III V	tt 4	36	77

ble III *Syndromes with bilateral congenital resia of the external auditory canal*

ndibulofacial dysostosis
anofacial dysostosis
ulooriculovertebral dysplasia
romosome 18 long arm deletion syndrome
minant craniometaphyseal dysplasia
crotia, meatal atresia, and conduction hearing loss
icrotia, hypertelorism, facial clefting, and conduction earing loss
r malformations, cervical fistulas or nodules, and mixed earing loss
resia of the external auditory canal and conduction deafness
iedocranial dysplasia
ypophthalmus syndrome and mixed deafness
aget' disease of bone
enal, genital, and middle ear anomalies
op ears, micrognathia, and conduction deafness

rom B. W. Konigsmark & R. J. Gorlin 1976. *Genetic and Metabolic Deafness* W. B. Saunders company Philadelphia, London Toront

Normal position of the external auditory canals. No cervical cysts or fistulas No preauricular pits No known heart or kidney defects No mental retardation Hands not examined.

From the age of 2 months he received orthopedic treatment for his foot deformities and was operated on bilaterally at the age of 2 years with good results At 6 years of age he was submitted to our clinic X-ray tomography revealed symmetrically narrowed external auditory canals At the subsequent operation of the right ear the middle ear was found normal except for a malleus fixation The meatal stenosis was only slightly less pronounced than the subtotal atresia found when operating on his cousins Present hearing loss TI right 25 dB TI left, 35 dB Normal bone conduction Normal karyotype

DISCUSSION

The collection of data concerning this family has been rather difficult and is not entirely satisfactory as a consistent examination of all family members has not been possible One reason is that many of the family members have been extremely reluctant to pass on information which seems to be due to a strong tendency within the family not to recognize or accept the family disease Although this might be quite understandable one consequence has been that none of the three affected boys received any audiological treatment before school age This is not acceptable in Denmark

Although the material is therefore not altogether satisfactory there is no doubt that the family represents an hereditary syndrome This is primarily characterized by the finding of congenital bilateral symmetrical and isolated subtotal atresia of the external auditory canal in all of the 6 affected descendants of the affected grandfather

The incidence of moderate to severe forms of congenital atresia of the external auditory canal has been estimated as 1 in 5 to 20 000 live births (Jafek et al 1975) Of 311 examined atretic auditory canals these authors found only 9 to be isolated 29% of the patients examined had bilateral atresia. Judging from this American material a cautious estimate would rate the incidence of the present atresia to be around 1 in 1 000 000 live births.

Secondly 4 out of the 6 affected descendants have bilateral foot anomalies—two cousins having congenital vertical talus Based on a Danish material (Becker Andersen & Reimann 1974) the incidence of congenital vertical talus is 1 in 10 000 live births as only 19 patients were recorded with this deformity over a 10-year period out of approximately 20 000 live births a year covered by their clinic

Finally all of the 3 affected boys in the third generation have an increased interocular distance of the same magnitude though the increase is not sufficient to be defined as ocular hypertelorism

We find the full syndrome to consist of

1 congenital bilateral symmetrical and isolated subtotal atresia of the external auditory canal

– bilateral congenital vertical talus (or possibly other bilateral foot-deformity)

3 increased interocular distance although not ocular hypertelorism.

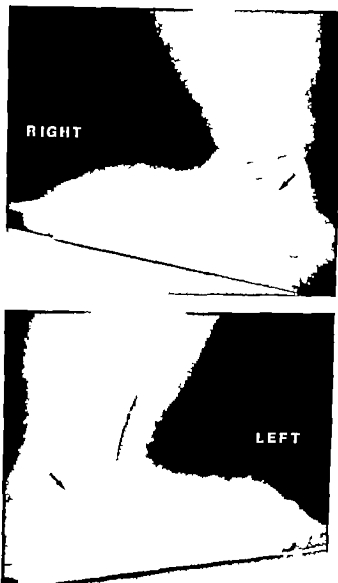


Fig 5 The feet of III 15 at the age of 16 months. (Arrows indicate vertical talus.)

dermatoglyphics (Table II) Normal position and appearance of the pinnae Normal position of the external auditory canal No cervical cysts or fistulas No preauricular pits IgA normal No known heart or kidney defects No mental retardation

He underwent surgery for the talus verticalis at the age of 3 months and later at the age of 6 years with moderate effect At the age of 7 he was submitted to our clinic as his hearing impairment was immediately recognized when beginning at school Tomography of the temporal bones revealed both external ear canals to be almost obliterated by a 3 mm wide bony formation the external auditory canals measuring 2 mm on the right ear 1.5 mm on the left (Fig 4) No other anomalies were revealed by the X ray examination. His right ear was operated twice at the age of seven The middle ear appeared perfectly normal Present hearing loss TI right 24 dB, TI left 45 dB Normal bone conduction. Normal karyotype

III 2 Normal hearing normal feet. Otherwise not examined

III 3 A 10-year-old boy with congenital bilateral atresia of the external auditory canal, pyloric stenosis increased interocular distance (Table I) bilateral clinodactyly and bilateral single transverse palmar crease (Fig 2c and Table II) Dermatoglyphics otherwise rare though normal Normal appearance and position of the pinnae Normal position of the external auditory canal No cervical cysts or fistulas No preauricular pits No known heart or kidney defects No mental retardation.

At the age of one month operated on for pyloric stenosis No subsequent problems. As in the case of the older brother he was also submitted to our clinic at the age of 7 after having started school His right ear was operated on once at the age of 7 after confirmation by X ray tomography of a symmetric subtotal atresia of the external auditory canals being approximately 2–3 mm wide At the operation a normal middle ear was found, except for a slight deformity of the manubrium mallei Present hearing-loss TI right 30 dB; TI left 45 dB Normal bone conduction Normal karyotype

III 4+6 A 20-year-old and a 15-year-old male with severe mental retardation Both had been thoroughly examined as children without finding any physical defects The cause of the mental retardation has not been established

III.5 7 8 9 10 11 12 13 and 14 All are reportedly normal

III 15 An 8-year-old boy with congenital bilateral atresia of the external auditory canal congenital bilateral talus verticalis (Fig 5) and increased interocular distance (Table I) Normal appearance and position of the pinneae

CONGENITAL UNILATERAL LOWER LIP PALSY

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Abstract Thirty-nine cases of congenital facial asymmetry in which one corner of the mouth does not dip downward symmetrically with the other are presented. The author has termed this condition congenital unilateral lower lip palsy (CULLP). CULLP was the most frequent condition in congenital facial anomalies seen in our clinic Complaints were purely cosmetic. No functional disorders in pronouncing labial sounds or in suckling were noticed A for pathogenesis, an insufficiency of the unilateral depressor labii inferior muscle is the most likely cause An important point is that the lower lip when drawn down is normal whereas the contralateral lip is paralysed As far as facial asymmetry is concerned CULLP is minor deformity Nevertheless possible association with other anomalies must be seriously considered when examining patient especially neonates As for treatment, selective facial nerve blocking is successfully done in our clinic

Congenital asymmetry of facial movement in neonates is rather infrequent and has a variety of causes Among the children referred to our clinic for evaluation of congenital facial paralysis it was noted that a number did not show the typical features of either a central or complete peripheral paralysis of the face They had a very limited facial palsy of congenital origin. This special type of palsy has been reported in Japanese journals by the present author as a special entity called congenital unilateral lower lip palsy CULLP (Kobayashi 1974 1975) This condition has not received much attention in otolaryngology thus far

DEFINITION AND REVIEW OF LITERATURE

CULLP is a minor congenital anomaly affecting the muscle normally employed to draw down the unilateral lower lip This condition does not appear to be associated with any *evidence of birth trauma*

Marino (1953) described paralysis of the muscle of the chin in the adult The most frequent cause in his cases was section of the facial nerve branch during incision and drainage for abscess of the angle of the mandible Other surgery included radical neck dissection and submaxillary gland removal He also observed obstetric poliomyelitic and cryptogenic cases Hoefnagel & Penry (1960) reported six cases with a characteristic form of congenital facial paralysis due to unilateral weakness of the lower lip depressor muscles Subsequently Cayler (1969) McHugh (1969) Verger et al (1970) Pape & Pickering (1972) Nelson & Eng (1972) Perlman & Reisner (1973) and Papadatos et al (1974) reported the same condition using different nomenclature

CASE MATERIAL

For the last 8 years (1969–77) 39 cases of CULLP have been seen at our facial nerve clinic During this period 43 cases of congenital anomalies of facial movement were encountered CULLP was the most frequent Other known entities were birth trauma, Moebius syndrome and Bonnevie-Ullrich syndrome The remainder did not fit into any known entity (Table I)

The patients ranged in age from months to 54 years Twenty-two cases were male and 17 cases were female Twenty-four patients had CULLP on the left side 15 on the right. In all

The other congenital defects described within this family must at present be considered to be coincidental

The mode of inheritance is most likely autosomal dominant with variable expressivity A recessive mode of inheritance is unlikely due to the rarity of the described traits An X linked dominant mode of inheritance seems ruled out by the fact that one daughter in the second generation is reportedly normal but this could be explained by variable expressivity

Because of the distinct features of the described syndrome and lack of features suggesting relationship to other known syndromes with bilateral congenital atresia of the external auditory canal (Table III) we find this syndrome to be yet another contribution to possible later identification of the chromosomal sites of genes governing the development of the human being

The presentation of this syndrome finally serves as a reminder of the importance of thorough examination of the entire family as well as of the proband with a congenital malformation

ACKNOWLEDGEMENTS

Dermatoglyphics and karyotyping using the reverse banding technique was performed by Dr Erik Niebuhr and his staff at the University Institute of Medical Genetics, Copenhagen His help and advice is very much appreciated Karyotyping using the G-banding technique was performed by Prof John Philip Div of Clinical Genetics Dept of Obstetrics and Gynecology YA and Dept of Pediatrics, Rigshospitalet, Copenhagen. Dr E. Hjalmar Larsen of the Orthopaedic Hospital Copenhagen kindly supplied us with literature and records concerning these patients with congenital vertical talus

ZUSAMMENFASSUNG

Von 20 Nachkommen eines Mannes der angeblich unter einer Ohr und Fußkrankheit gelitten hat, leiden 6 unter einer kongenitalen bilateralen, symmetrischen der isolierten subtotalen Atresie des äußeren Gehörgangs. Vier von diesen 6 Nachkommen haben dazu bilaterale Fußanomalien und von diesen 4 haben 2 (Vetter) kongenital vertikal talus In der 3 Generation haben die 3 befallenen Knaben vergrößerten Interokularen Abstand. Obwohl under den Nachkommen einige Fälle mit kurzen 5 Finger bilateraler einzeltransversaler Handbeugefurche pylorus stenose und kongenitaler Esotropie gefunden worden sind, werden diese als zufällig zusammenfallende Befunde beobachtet Bei den befallenen Nachkommen sind – abgesehen von den Ohratresien – sowohl die ORL-Untersuchung als auch der mentale Zustand, der Fingerabdruck IgA und die Nieren- und Herzfunktion normal Die Karyotype der 4 befallenen Nachkommen die untersucht worden sind, waren normal. Ein autosomal dominanter Erbgang mit variabler Expressivität könnte hier vorliegen

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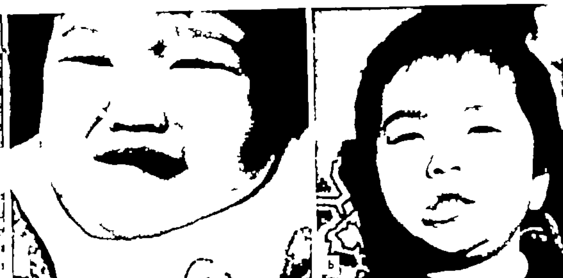


Fig. 1. Congenital unilateral lower lip palsy (a) Right-sided palsy (*b*) left-sided palsy

datos et al (1974) By contrast Cayler s cases (1969) from a cardiac clinic were predominantly right sided According to Perlman the frequency of associated major congenital anomalies cardiac and others may be related to the side of the lesion

There was no significant sex difference in any of the studies

Pathology

Which muscle is affected in CULLP? There are four muscles in the sublabial area. M orbicularis oris M depressor anguli oris M depressor labii inferior and M mentalis (Fig. 2) These muscles are innervated by the marginalis mandibulae branch of the facial nerve Hoefnagel & Penry (1960) speculated that this condition was due to a unilateral weakness of the mentalis and the depressor labii inferior McHugh et al (1969) reported weakness of the depressor anguli oris muscle in electrodiagnosis However no reporters proved their speculations by biopsy or surgery The present author concludes from visual inspection of patients appearance and electromyography that the insufficiency of the depressor labii inferior muscle plays the most important role However he does not deny that the insufficiency of the other muscles might play some parts in CULLP Fig 3 shows the apparent difference in muscle activity between the two sides

There is a variety of degrees of congenital facial asymmetry CULLP is the most limited



Fig 2 Anatomy of the lower face (Hollinshead)

Table I *Statistics from Facial Nerve Clinic University of Tokyo Department of Otolaryngology (Jan 1970 to Dec 1977)*

Bell's palsy	254
Hunt s syndrome	67
Trauma	
Accidental	51
Surgical	71
Otitis media	
Acute	4
Chronic	27
Neonatal	
CULLP	39
Birth trauma	
Bonnevie Ullrich syndrome	1
Möbius syndrome	3
Others	5
Polyneuritis	7
Tumor	
VII nerve	5
VIII nerve	5
Intracerebral	3
Parotid	6
Leukemia	3
Others	3
Hypercalcemia	
Etiology unknown	34
Hemifacial spasm	101
Others	29
Total	722

cases the abnormality was noticed at birth There were no difficulties in suckling in early infancy

No feto-toxic drug intake or maternal rubella was noted during pregnancy There were no diffidult deliveries In no cases were obstetrical forceps used No hereditary factor was found

Concerning the presence of other anomalies (Table II) there were 18 cases of ear anomalies There were 3 cases of cardiac defects 2 of delayed speech development and 2 of abnormal minor neurological signs including ipsilateral hemifacial hyperhidrosis contralateral pyramidal tract signs anisocoria and abnormal EEG finding Other abnormalities found in our cases were congenital dislocation of the hip torticollis scoliosis webbed neck pes valgus and small penis

One case underwent chromosomal analysis with normal result

DISCUSSION

Complaint

Patients chief complaint was an inability to draw down the lower lip unilaterally At rest position facial asymmetry was not noticeable but became evident particularly when crying and laughing. Hence the term asymmetric crying face was reported by Pape & Pickering (1972) Other mimetic movements such as forehead wrinkling eye closure and pouting were common An important point is that it is the drawn-down lower lip that is normal and the contralateral lower lip that is paralysed. Occasionally the physicians who referred patients to us misunderstood the involved side Fig 1 shows a typical appearance

Complaints were purely cosmetic No functional disorders in pronouncing of labial plosive sounds or suckling were noticed Adult patients were mostly young women possibly because males only slightly affected by CULLP ignored it

Frequency–side–sex

Perlman & Reisner (1973) found 41 cases of CULLP among 6360 newborns (an incidence of 0 6%) in Israel Papadatos et al (1974) detected 37 cases in 4530 neonates (an incidence of 0 8%) in Greece Our recent survey revealed 2 cases in 2071 newborns over a 12-month period in Yoneyama Maternity Hospital in suburban Tokyo The incidence in Japan was lower than abroad

In our cases the left sided palsy was more frequent This finding coincides with the reports of Perlman & Reisner (1973) and Papa-

Table II *Associated anomalies*

Ear anomaly	18
Cardiac defect	3
Abnormal neurological sign	
Delayed speech	
Congenital dislocation of the hip	1
Torticollis	1
Scoliosis	1
Webbed neck	1
Pes valgus	1
Small penis	1



Fig 5 ( ) Before the blocking (*b*) after the blocking

factor might be involved. However he favored a multifactorial etiology as the monozygous twins among his cases showed discordance

Association with other anomalies

In 20 out of 39 patients in the present series facial asymmetry coexisted with other anomalies. In three instances cardiac anomalies were found Cayler (1969) paid special attention to cardiac anomalies and described a "cardiofacial syndrome" According to Cayler the association might be related to the proximity of the hyoid arch to the cardiac primordium and/or to the chronologically close embryonic development of facial innervation and cardiac septation Further extended survey by Pape & Pickering (197?) revealed an association with urogenital musculoskeletal respiratory gastrointestinal and cardiac anomalies In this connection it is suggested that CULLP be used as an index for the presence of congenital malformations

Treatment

Treatment is rather difficult. If patients or their families do not mind no treatment is necessary In some children the cosmetic defect lessened with increasing age It is not clear whether this is due to conscious avoidance of crying and grimacing, which de emphasizes the imbalance or due to true improvement of muscle force However treat ment is important, especially for the young woman who is psychologically affected by her facial asymmetry Marino excised a portion of the marginal mandibular branch of the facial nerve of the unaffected side This is selective neurectomy Selective myectomy can also be done (Freeman 1964) Through a sublabial incision the depressor labii inferior muscle is excised.

We do a selective facial nerve branch block using evoked electromyography (Totsuka et al 1972) In most cases the block is per formed under sedation such as intravenously administered diazepam. With apprehensive patients however endotracheal anesthesia is employed (Figs 4 and 5)

Step 1 A pair of surface electrodes are attached in the infra-auricular region of the affected side A square wave electrical pulse of 3 msec in duration and of 50 to 80 V intensity is applied through the electrodes and the facial nerve trunk is stimulated percuta neously A minimal threshold of intesity for eliciting the contraction of the pertinent muscle is then determined A coaxial needle electrode is inserted into the pertinent muscle and the evoked EMG response is observed on an oscilloscope display

Step 2 As stimulating needle electrodes a pair of hypodermic needles insulated except for the tip are inserted at possible sites for the peripheral course taken by the nerve branch innervating the pertinent muscle A pair of needles is singled out and electrical

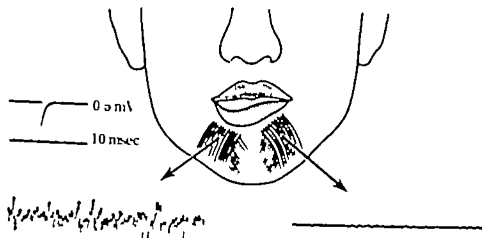


Fig 3 Electromyography of the depressor labii inferior muscles.

facial palsy that we have observed The most difficult point to understand is why the lower lip area is predominantly affected

Pathogenesis

The present author admits that he is ignorant of the true pathogenesis of this abnormality Marino (1953) reported cases due to obstetrical trauma However this trauma usually has a favorable prognosis and is therefore not likely to be the cause of CULLP which has an unfavorable prognosis

Congenital absence of a single muscle is not uncommon The pectoralis major gastrocnemius serratus posterior and abdominalis are often absent McHugh et al (1969) reported a rare case with the absence of the frontalis and orbicularis oculi muscles in which he did a biopsy but did not find any muscle fibers He described this as agenesis Nelson & Eng (1972) speculated that congenital hypoplasia of the depressor anguli oris muscle might provide an explanation for the entity currently under discussion

In the present cases anomalies of the external ears were found in 18 cases In one of these cases chromosomal analysis was performed and showed no abnormality However Cayler (1969) observed chromosomal aberration in three cases and postulated that CULLP might be due to subclinical viral infection occurring in the mother during the fifth week of pregnancy Though no abnormalities during pregnancy were identified by careful history taking in the present series some noxa might have influenced the fetus during the developmental period of the facial musculature In Thalidomide babies and maternal rubella syndrome maxillofacial anomalies involving the lower half of the face innervated by the marginal mandibulae branch are frequently found (Peet 1971 Nager 1971 d Avignon & Barr 1964)

Perlman & Reisner (1973) discussed the hereditary factor In his series three patients had an incidence of facial asymmetry in more than one family member Papadatos et al. (1974) observed that 13 out of 74 parents were affected and suggested that the hereditary

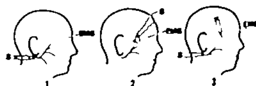


Fig 4 Selective facial nerve branch blocking. S. stimulator

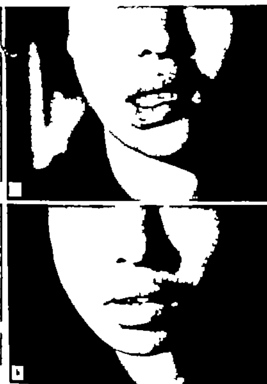


Fig 5 ( ) Before the blocking; (b) after the blocking.

factor might be involved. However he favored a multifactorial etiology as the monozygous twins among his cases showed discordance

Association with other anomalies

In 20 out of 39 patients in the present series facial asymmetry coexisted with other anomalies In three instances cardiac anomalies were found. Cayler (1969) paid special attention to cardiac anomalies and described a cardio-facial syndrome According to Cayler the association might be related to the proximity of the hyoid arch to the cardiac primordium and/or to the chronologically close embryonic development of facial innervation and cardiac septation Further extended survey by Pape & Pickering (1972) revealed an association with urogenital musculoskeletal respiratory gastrointestinal and cardiac anomalies In this connection it is suggested that CULLP be used as an index for the presence of congenital malformations

Treatment

Treatment is rather difficult If patients or their families do not mind no treatment is necessary In some children the cosmetic defect lessened with increasing age It is not clear whether this is due to conscious avoidance of crying and grimacing, which de-emphasizes the imbalance or due to true improvement of muscle force However treatment is important especially for the young woman who is psychologically affected by her facial asymmetry Marmo excised a portion of the marginal mandibulae branch of the facial nerve of the unaffected side This is selective neurectomy Selective myectomy can also be done (Freeman 1964) Through a sublabial incision the depressor labii inferior muscle is excised

We do a selective facial nerve branch block using evoked electromyography (Totsuka et al 1972) In most cases the block is performed under sedation such as intravenously administered diazepam. With apprehensive patients however endotracheal anesthesia is employed (Figs 4 and 5)

Step 1 A pair of surface electrodes are attached in the infra-auricular region of the affected side A square wave electrical pulse of 3 msec in duration and of 50 to 80 V intensity is applied through the electrodes and the facial nerve trunk is stimulated percutaneously A minimal threshold of intesity for eliciting the contraction of the pertinent muscle is then determined. A coaxial needle electrode is inserted into the pertinent muscle and the evoked EMG response is observed on an oscilloscope display

Step 2 As stimulating needle electrodes a pair of hypodermic needles insulated except for the tip are inserted at possible sites for the peripheral course taken by the nerve branch innervating the pertinent muscle A pair of needles is singled out, and electrical

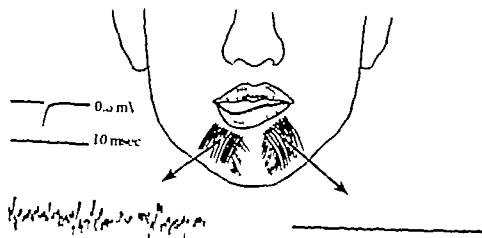


Fig 3 Electromyography of the depressor labii inferior muscles.

facial palsy that we have observed The most difficult point to understand is why the lower lip area is predominantly affected

Pathogenesis

The present author admits that he is ignorant of the true pathogenesis of this abnormality Marino (1953) reported cases due to obstetrical trauma. However this trauma usually has a favorable prognosis and is therefore not likely to be the cause of CULLP which has an unfavorable prognosis

Congenital absence of a single muscle is not uncommon The pectoralis major gastrocnemius serratus posterior and abdominalis are often absent McHugh et al (1969) reported a rare case with the absence of the frontalis and orbicularis oculi muscles in which he did a biopsy but did not find any muscle fibers He described this as agenesis Nelson & Eng (1972) speculated that congenital hypoplasia of the depressor anguli oris muscle might provide an explanation for the entity currently under discussion

In the present cases anomalies of the external ears were found in 18 cases In one of these cases chromosomal analysis was performed and showed no abnormality However Cayler (1969) observed chromosomal aberration in three cases and postulated that CULLP might be due to subclinical viral infection occurring in the mother during the fifth week of pregnancy Though no abnormalities during pregnancy were identified by careful history taking in the present series some "noxa" might have influenced the fetus during the developmental period of the facial musculature In Thalidomide babies and maternal rubella syndrome maxillofacial anomalies involving the lower half of the face innervated by the marginal mandibular branch are frequently found (Peet 1971 Nager 1971 d Avignon & Barr 1964)

Perlman & Reisner (1973) discussed the hereditary factor In his series three patients had an incidence of facial asymmetry in more than one family member Papadatos et al (1974) observed that 13 out of 74 parents were affected and suggested that the hereditary



Fig 4 Selective facial nerve branch blocking. S. stimulus

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stimulation is applied while observing the evoked EMG response If the tips of the needles are at some distance from the nerve branch the threshold for evoking the response will be high Successive pairs of needles are then selected and the corresponding thresh olds are determined When an evoked re sponse is obtained with a minimum intensity of stimulation it can be concluded that the tip of one of the electrodes being sampled is closest to the branch

Step 3 Approximately 2 ml of 5% phenol in glycerin is injected through chosen needle electrode during continued observation of evoked responses on an oscilloscope display If the block is effective the amplitude of the evoked response will gradually diminish and finally disappear when electrical stimulation is again given via surface electrodes to the nerve trunk.

Diagnosis differential diagnosis

CULLP is easily recognizable in newborns The asymmetry is most conspicuous on cry ing EMG study shows diminished voluntary activity Electrical silence or sparse low volt age units are detected in the depressor labii inferior muscle No neurogenic pattern is ob- served According to Nelson & Eng (1972) nerve excitability may be elevated and con duction latency may be prolonged

Central nuclear and peripheral facial palsy due to a variety of causes should always be differentiated Other important diseases which should be ruled out are macrostomia first and second arch syndrome and Treacher Collins syndrome

Terminology

This condition has been given a variety of names in the earlier literature Since no defi- nite conclusion has been made concerning the defective muscle the author feels that con- genital unilateral lower lip palsy (CULLP) is the most suitable at the present time In CULLP the word palsy includes not only genuine palsy of the nerve but muscle insuffi- ciency in a broad sense In this connection the term congenital unilateral lower lip insuffi- ciency syndrome might also be employed.

ACKNOWLEDGEMENT

The author is grateful to Drs T K'kawada, M Ch and M Higurashi University of Tokyo and Dr A S U S Naval Regional Medical Center at Yokosuka their valuable help in this study

ZUSAMMENFASSUNG

Neununddreißig Fälle mit angeborener Gesichtsas metrie wurden dargestellt bei denen sich ein Mundwi nicht symmetrisch mit dem andern abwärts bewegt Autor bezeichnete diesen Zustand als „angeborene seitige Unterlippenlähmung (CULLP)" CULLP w häufigste Fall bezüglich Gesichtsanomalien der in uns Klinik beobachtet wurde Beschwerden waren rein metischer Art Funktionelle Störungen bei der sprache von Lippenlauten oder beim Milchsaugen wu nicht beobachtet Bezüglich der Pathogenese ist Insuffizienz des einseitigen M depressor labii info wahrscheinlich Wichtig ist daß die herunterge Unterlippe normal und die gegenüberliegende Li paralysiert ist CULLP stellt soweit es die Ges asymmetrie betrifft, eine geringfügige Mißbildung Mögliche Assoziationen zu anderen Anomalien wer jedoch ernsthaft bei der Untersuchung on Patien insbesondere Neugeborenen, in Erwägung gezogen. Behandlung w rd in unserer Klinik erfolgreich selektiv Blockieren von Gesichtsnerven durchgeführt

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Table I *The values of the maximum resting tone of the pharyngo-oesophageal sphincter together with the extent of the sphincter in normal and laryngectomized patients respectively*
The patient numbers refer to Table II

	Normal values Mean ± 1 S.D. 8 normal subjects	Individual values of 3 laryngectomized patients		
		No 10	No 12	No. 15
Maximum resting sphincter tone	22.4±9 6 mmHg	3.2 mmHg	7 6 mmHg	4.8 mmHg
Extent of sphincter region	3±1.3 cm	3.8 cm	4 8 cm	2.5 cm

METHOD

Both the patients and the volunteers were subjected to manometric investigation of the pharyngo-oesophageal sphincter In addition the patients were given a voice test

The pressure measurements were carried out with a continuously perfused low-compliance system with three side holes corresponding to the pressure registering parts of the catheter In the normal subjects registration was carried out of the maximum resting tone of the sphincter as well as of the extent of the sphincter region. The corresponding sphincter parameters were also registered in the patients as far as it was possible to demonstrate a high pressure zone corresponding to the pharyngo-oesophageal sphincter Each parameter was registered five times. The pressure measurements were carried out as described by Roed Petersen (1978)

All the patients took part in the voice test, which consisted of reading of a standard text containing 220 syllables The reading was recorded on tape with registration of the reading speed. The intelligibility of the tape recording was evaluated by all three of the present authors using the following grades based on the quality and having no regard to the speed of reading: good (IV) middle (III) poor (II) unintelligible (I)

RESULTS

A well defined zone with increasing resting tone corresponding to the pharyngo-oesophageal sphincter was registered in all the normal subjects The normal values calculated as mean values and standard deviation based on the mean values of the individual subjects are shown in Table I

It was only possible to register a sphincter tone in 3 of the 17 laryngectomized patients the values for these 3 patients are also shown in Table I In the remaining 14 patients a resting pharyngo-oesophageal sphincter pressure was recorded similar to the intra-oesophageal pressure

The results of the voice test are shown for the individual patients in Table II and at the same time the patients using a vibrator are indicated as well as those troubled by dysphagia.

DISCUSSION

As stated in the survey of Diederich & Youngstrom (1966) of alaryngeal speech the generally accepted hypothesis is that the neoglottis in laryngectomized patients is situated in the pharyngo-oesophageal sphincter However this hypothesis was first rendered probable following electromyographic studies carried out by Shipp in 1970

Shipp (1970) studied 18 laryngectomized patients by measuring the activity of the inferior pharyngeal muscle during air insufflation and during phonation During glossopharyngeal air injection which was employed by 17 of the patients muscle activity was registered which could be interpreted as an ex

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THE PHARYNGO-OESOPHAGEAL SPHINCTER AFTER LARYNGECTOMY

A Manometric Investigation

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Abstract The function of the pharyngo-oesophageal sphincter in patients subjected to total laryngectomy has been evaluated by manometric measurement and the results related to the patient's ability to speak with an oesophageal voice as well as to the occurrence of dysphagia. Seventeen totally laryngectomized patients were studied The intelligibility of the patient's oesophageal voice was classified according to the scale good (group IV) middle (group III) poor (group II) and unintelligible (group I) The manometric investigation was carried out with a continuously perfused low compliance system with three side holes, and the results of the patient investigation were compared with those from a normal material It was possible in three patients only to demonstrate a resting tone corresponding to the pharyngo-oesophageal sphincter The pressure was lower in these patients than in the normal material No correlation was found between any of the three parameters, sphincter pressure intelligibility and the presence of dysphagia.

The importance of the pharyngo-oesophageal sphincter for the development of an intelligible oesophageal voice in patients subjected to laryngectomy has been previously studied in a number of investigations by means of radiography In addition a few investigations have been reported during recent years concerning manometric measurement of the sphincter region in these patients (Reichbach et al 1970 Sandberg 1970 Månsson & Sandberg 1974) Attention has also been given to the possibility that the dysphagia which can occur in laryngectomized patients can be attributed to a dysfunction of the sphincter (Schobinger 1958 Sandberg 1970)

The object of the present investigation has been to determine whether it is possible to demonstrate by means of manometric me urement a correlation between the rest tone of the sphincter the patient's ability develop an intelligible oesophageal voice the possible presence of dysphagia.

MATERIAL

The total material comprises 20 patients during the period 1971 to 1977 were subjec to laryngectomy following primary radia therapy for cancer of the larynx Two of patients were unable to complete the ma metric examination owing to dyspnoea bronchial asthma, respectively a further tient refused to take part in the investiga The average age of the remaining 17 patie (all men) was at the time of investigati 61 years (range 19–74 years) and the aver period of observation 3 years and one mo (range 6 months – 6½ years) Twelve of patients had developed the ability to use oesophageal voice while the other 5 patie had to use a vibrator Two of the patie were inconvenienced by pharyngo-oesop geal dysphagia with a feeling of food stick in the throat but without this giving rise any loss of weight Both of these patients u a vibrator

The normal material consisted of 8 vol teers (6 men and 2 women) having no gas oesophageal disease their average age was years (range 51–67 years)

-tion to the normal material were included in the groups II III and IV The patient in group II used a vibrator Of the 14 patients in the material who had no demonstrable resting tone, 4 used a vibrator the distribution of these 4 patients was 3 in group I and one in group II Of the remaining 12 patients who were able to produce an intelligible oesophageal voice 8 belonged to group IV 3 to group III and one to group II Thus the present work does not differ from the other manometric investigations with regard to the results of the pressure measurements inasmuch as we also find that the pharyngo-oesophageal sphincter tone is reduced or absent after laryngectomy On the other hand we find no correlation between pressure and the quality of the oesophageal voice or the lack of ability to develop such a voice This must be interpreted in the following manner that a low pressure in the sphincter is an enhancing element but not a sufficient basis for the patient to learn to speak with an oesophageal voice It must be accepted on the basis of the work of Shipp (1970) that patients are able to develop voluntary control of the pharyngo-oesophageal sphincter which is of importance for the development of the voice Thus a low sphincter pressure and the ability to develop voluntary control of the sphincter are prerequisites of an intelligible oesophageal voice

In our material 2 of the patients (12%) were inconvenienced by dysphagia Schobinger (1958) found in comparison a frequency of 24% among 42 patients while Sandberg (1970) had a frequency of 5% (one patient of 20) This patient suffered from dysphagia and had manometric signs of pharyngo-oesophageal sphincter spasm Furthermore he was unable to speak with an oesophageal voice Sandberg (1970) concluded that difficulty in swallowing occurring after laryngectomy appears to be of lesser importance with regard to the patient s nutritional condition, but that the basic disturbance in the swallowing mechanism can explain why the patient is unable to develop an intelligible oesophageal voice We found in agreement with Sandberg (1970) that the 2 patients who were troubled by dysphagia both belonged to group I but in contrast to Sandberg s patient ours had practically no sphincter tone We are thus unable to confirm the theory of a causal relationship between dysphagia and absence of the ability to develop an oesophageal voice as a result of sphincter spasms

ZUSAMMENFASSUNG

Mittels einer „low-compliance"-Dreipunktmanometrie mit kontinuierlicher Perfusion wurde die Funktion des pharyngoösophagealen Sphinkters in 17 total laryngektomierten Patienten beurteilt anhand einer normalen Kontrollgruppe. Außerdem wurden Dysphagiehäufigkeit und Verständlichkeit der erworbenen Oesophagussprache den Ergebnissen der Druckmessungen gegenübergestellt. Die Verständlichkeit der alaryngealen Sprache wurde wie folgt unterteilt gut verständlich (Gruppe IV) mittelmäßig (Gruppe III) schlecht (Gruppe II) und nicht verständlich (Gruppe I) Bei nur drei der Patienten ließ sich ein Ruhetonus nachweisen diese Druckwerte waren wesentlich niedriger als die der Kontrollgruppe. Es konnte kein Zusammenhang zwischen den drei Parametern Sphinkterdruck Verständlichkeit der alaryngealen Sprache und Dysphagiehäufigkeit nachgewiesen werden.

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Table II *The intelligibility of the oesophageal voice of 17 totally laryngectomized patients divided into four groups (as described in the text)*

The numbers of syllables per second in those cases where the voice was intelligible whether the patient used a vibrator or whether they suffered from dysphagia are also shown

Pat no	Intelli-gibility group	No of syllables per second	Vi-brator	Dys-phagia
1	IV	4..		
2	IV	2.5		
3	IV	9		
4	IV	3.2		
5	IV	2.6		
6	II	3 0	+	
7	IV	2		
8	III	1 7		
9	I	–	+	+
10	III	2 0		
11	I	–	+	+
1	II	1 1	+	
13	III	1 6		
14	IV	.7		
15	IV	1 9		
16	II	0		
17	I	–	+	

pression of either distension during air insufflation or as being caused by muscle contraction The contraction could occur either at the same time as the glossus pressure was performed or afterwards in such a manner that the contraction in the latter case involved a proximal closure of the air column A single patient insufflated air by swallowing during which time an electromyographic pattern was registered corresponding to ordinary swallowing During phonation contraction complexes occurred which showed considerable variation between the individual patients but the main principle was still present that the patients with a good oesophageal voice were able to carry out a more differentiated contraction than patients with a poorly developed voice

The importance of the pharyngo-oesophageal sphincter with regard to alaryngeal speech has also been discussed by Samuel & Adams (1970) who using x ray examination demonstrated that after one syllable has been spoken a segment of the distal oesophagus collapses at the same time as the diaphragm is elevat and the neoglottis closes This is repeatec average three times after which the patient to fill the oesophagus with air These inv gations thus support the assumption that pharyngo-oesophageal sphincter has an n pendent function during alaryngeal speech

Sandberg (1970) and Månsson & Sand (1974) found by using manometric meas ments with a fluid filled non perfusing cath and intermittently perfusing catheter resp tively that the resting tone of the pharyn oesophageal sphincter was reduced postop tively in relation to the preoperative va However one of Sandberg s patients suff from spasms of the pharyngo-oesopha sphincter postoperatively This patient not been able to learn to speak with an o phageal voice the cause being considere be spasms of the sphincter

Reichbach & Winans (1970) studie 13 yngectomized patients manometrically means of a continuously perfused sys The authors found by comparing these tients with a normal material that the res tone of the pharyngo-oesophageal sphin was significantly reduced postoperatively also that patients with a good oesopha voice had a significantly lower sphin pressure than patients with a poor oesopha voice The authors explained this as b dependent on that air intake which they c sidered as being the most difficult part of ryngeal speech was easier to carry out a low sphincter pressure This is in full ag ment with the fact that air intake occurs n frequently by means of inhalation or injec (glossal or glossopharyngeal pressure) aga a passive sphincter and only in rare c does this take place by swallowing du which the sphincter relaxes (Diederich Youngerstrom 1966)

Only three of the laryngectomized pati in the present material had a demonstr resting tone corresponding to the pharyn oesophageal sphincter These 3 patients whom the sphincter tone was reduced in n

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THE VASCULAR SUPPLY OF THE ENDOLYMPHATIC SAC

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Abstract. The vascular anatomy of the endolymphatic sac in guinea pigs was examined following intravascular injection of silicone rubber (Microfil). Methacrylate resin of low viscosity (Mercox) was used to obtain vascular corrosion casts for scanning electron microscopy, which allowed more accurate differentiation between arteries and veins. The extensive vascular system around the sac comprises both arteries and veins as well as lymphatic vessels. The arterial supply is derived mainly from the posterior meningeal artery in the posterior cranial fossa. In some cases a small artery also leads to the sac from the posterior vestibular artery in the labyrinth (in 7 of the 35 animals investigated). It courses together with the vein of the vestibular aqueduct along the walls of the endolymphatic duct. The blood is drained over the intermediate portion of the endolymphatic sac which becomes lodged in a rich meshwork of capillaries, venules, veins and a few small arteries. A few venous trunks from both sac walls fuse with the vein of the vestibular aqueduct, which drains blood from the vestibule to the sigmoid sinus. Scanning electron microscopy also revealed numerous anastomosing vessels within bone channels with adjacent bone marrow sinusoids, which also probably contribute to the vascular supply of the endolymphatic sac.

Although the vascular structure of the inner ear has been studied extensively (Eichler, 1892; Siebenmann, 1894; Shambaugh, 1903; Nabeya, 1924; Smith, 1954; Axelsson, 1968; Hansen, 1971), the blood vessels of the endolymphatic sac have received scant attention. It was previously believed that the endolymphatic sac was only surrounded by venous ramifications, anastomosing on the one hand with the labyrinthine vessels and on the other with the sigmoid sinus through the vein of the vestibular aqueduct.

In human embryos the endolymphatic sac is ensheathed by a [illegible] plexus which becomes resolved into a few main channels connected with branches of the original network (Streeter, 1916). The most constant channel to have developed through the plexus is the vein of the vestibular aqueduct, which is present in most mammals. This vein and the inferior cochlear vein provide dual venous drainage of the labyrinth. In man the vein of the vestibular aqueduct collects blood from the utricle and the semicircular canals (Fig. 1, from Siebenmann, 1894) and runs separately from the endolymphatic duct in a bony canal originally named the *accessory canal of the vestibular aqueduct* (Fig. 2A, B, C). In recent years this canal has been called the paravestibular canal (PVC) (Ogura & Clemis, 1971; Wilbrand et al., 1974; Stahle & Wilbrand, 1974) and has been shown to contain a minute artery (Ogura & Clemis, 1971; Stahle & Wilbrand, 1974).

Obstruction of the labyrinthine artery causes rapid disintegration of the organ of Corti as well as histopathological changes in the vestibular membranous labyrinth (Perlmann et al., 1959). The endolymphatic sac, however, remains intact, except for its liquid contents, which become more proteinaceous and acidophilic. The reason for this is believed to be that this portion of the labyrinth receives tributaries from the posterior meningeal artery (PMA) in the posterior cranial fossa (Bast &

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Fig 2A Plastic mould Human right ear The accessory canal (*AC*) starts superiorly to the proximal end of the vestibular aqueduct (*VA*) (The bony labyrinth has been macerated and filled with plastic medium) Abbreviations. *SSCC* superior semicircular canal *PSCC* posterior semicircular canal *LSCC* lateral semicircular canal *GG* geniculate ganglion, *FC* facial canal, *CC* common crus, *CA* cochlear aqueduct.

absolute ethanol and finally cleared in methyl salicylate (wintergreen oil) The bones were then inspected in a stereomicroscope (Wild-Heerbrugg M5) and photographed in a light microscope (Wild Heerbrugg M20)

Micro-cast preparation

To distinguish arteries from veins a further 20 guinea pigs were perfused with methacrylate resin of low viscosity (Mercox Japan Vilene Company Ltd Tokyo Japan) to obtain vascular corrosion casts for scanning electron microscopy (SEM). Except for slight modifications the procedure was the same as described by Hodde et al (1977) and as given in the general instructions from the Mercox manufacturer To ascertain that the fixation was satisfactory for proper imprinting of the corrosion cast, at least 200 ml of 2.5% glutaraldehyde solution in 0 1 M Sörensen s phosphate buffer pH 7 4 was injected and to prevent vasoconstriction, a solution of 2% procaine chloride was also injected prior to the perfusion with methacrylate resin. In order to study the degree of vascular anastomosis with the surrounding bone vessels the petrous bone around the sac was macerated in 20% KOH but otherwise kept intact After maceration the preparations were rinsed in redistilled water air-dried and covered with gold in a Polaron SEM coating unit E 5000 at 1.2 kV and 10 mA for 4 min. Finally the specimens

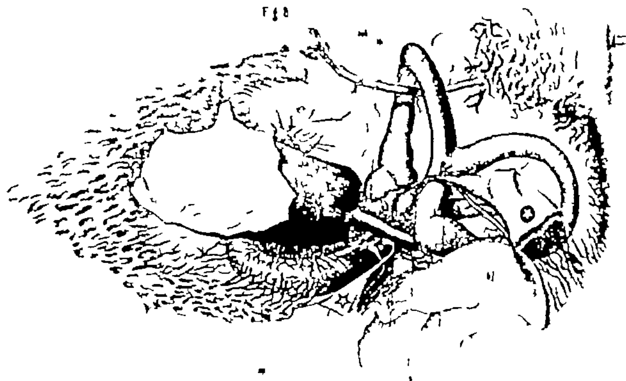


Fig 1 Drawing of a right human bony labyrinth demonstrating the vestibular (O) and cochlear (☆) aqueducts and their accessory canals (from Siebenmann, 1890)

Anson 1949) In the guinea pig (Smith 1953) as well as in the pig embryo (Shambaugh 1903) a small artery which branches from the posterior vestibular artery (PVA) in the labyrinth and courses with the endolymphatic duct inside the vestibular aqueduct has also been described

As there is reason to believe that the endolymphatic sac plays a significant part in the normal inner ear metabolism and that disturbances of its function may lead to labyrinthine disorders it would seem of crucial importance to examine its vascular anatomy and to determine whether and if so how it is supplied arterially with blood This may also have implications during various surgical operations on the endolymphatic sac (Arenberg et al 1977)

The present investigation was therefore undertaken to study the vascular system of the endolymphatic sac

MATERIAL AND METHODS

Silicone rubber (Microfil) injection

Thirty five healthy pigmented guinea pigs weighing 200 to 300 g were used They were anaesthetized with sodium pentobarbital (Nembutal) and heparinized through the femoral vein The thoracic cavity was opened and the aorta was clamped above the diaphragm A cannula was then inserted through the left ventricle and the vascular system was perfused with warm saline solution until the fluid flowing out through the right atrium became clear Microfil 122 (Canton Biomedical Products Boulder Colo USA) was then infused into the ascending aorta at a pressure of 25–150 mmHg The iridic capillaries were inspected to check whether the filling was complete The temporal bones were removed and fixed overnight in 10% formalin dehydrated through a series of graded ethanols and



Fig. 2C. Plastic mould of right human labyrinth, anterior view. Several small channels (*black arrows*) drain some of the vestibular blood through the vein of the vestibular aqueduct, which courses within the accessory canal of the vestibular aqueduct. This canal is seen medial to the aqueduct (*VA*). For other abbreviations, see Fig. 2A.

different regions, each vascular area was then related to the length of the region rather than to its subepithelial tissue area, and mean values for the different regions were calculated.

FINDINGS

Vascular injections

By dehydration of the soft and bony tissue and clearing in wintergreen oil the specimens became partially transparent and the three-dimensional outline of the vascular tree was clearly defined. The specimens were filled to various degrees. Some showed complete capillary filling, but most of them were incompletely filled. One striking observation was that despite incomplete injection in some cases, with total lack of intralabyrinthine filling, the vessels of the endolymphatic sac were often well outlined. At SEM of vascular corrosion casts the surface imprints of the arteries and veins were seen to have a typical pattern (Figs. 4A–F and



Fig 2B Plastic mould of a right human labyrinth Inferior view illustrating the peripheral widening of the vestibular aqueduct The accessory canal (*AC*) joins the aqueduct at its intermediate portion For other abbrevations see Fig. A

were examined in a scanning electron microscope (JEOL JSM U3)

Histological sectioning

Ten guinea pigs were perfused with 2% glutaraldehyde and 1% formaldehyde in 0 1 M Sörensen s phosphate buffer pH 7 4 850 mosmol and 0 05 M Na-cacodylate buffer pH 7 4 830 mosmol In 5 animals the temporal bones were decalcified and fixed in a solution of 0 1 M Na EDTA 2 5% glutaraldehyde and 0 05 M Na-cacodylate pH 7 2–7 4 900 mosmol for 2 months while in the other 5 animals the entire sac was removed from the petrous pyramid and immersed in the perfusion fixative for 24 h post fixed in 1% osmium teroxide dehydrated in alcohol and propylene oxide embedded in Epon 812 (Luft 1961) trimmed and sectioned on an LKB Ultrotome. Serial sections of the sac were made both perpendicular and parallel to its longitudinal axis by rough cutting (sections 1–? μm) Every 10th section was stained for light microscopy with toluidine blue or 1% paraphenylenediamine Ultrathin sections (500–800 Å) were also made and contrasted with uranyl acetate and lead citrate and examined in JEOL 100 B electron microscope In order to get an idea of the vascular density in the different parts of the sac the vascular area was then calculated on histological sections by means of a mensural ocular and an optical scale (1/100 mm). Differences in the epithelial morphology served as guidance for the subdivisions of the sac Owing to the large differences in the width of the perisaccular connective tissue in the



Plastic mould of right human labyrinth, ...ew. Several small channels (*black arrows*) ...ne of the vestibular blood through the ...ein of ...ibular aqueduct, which courses within the accessory canal of the vestibular aqueduct. This canal is seen medial to the aqueduct (*VA*). For other abbreviations, see Fig. 2 A

...nt regions each vascular area was then ...l to the length of the region rather than ...ubepithelial tissue area and mean values ...: different regions were calculated

FINDINGS

...ular Injections

...hydration of the soft and bony tissue and ...ing in wintergreen oil the specimens be... partially transparent and the three-dimensional outline of the vascular tree was clearly defined. The specimens were filled to various degrees Some showed complete capillary filling, but most of them were incompletely filled. One striking observation was that despite incomplete injection in some cases, with total lack of intralabyrinthine filling the vessels of the endolymphatic sac were often well outlined At SEM of vascular corrosion casts the surface imprints of the arteries and veins were seen to have a typical pattern (Figs. 4*A*–*F* and

5) which allowed accurate differentiation between the two types of vessels. The arteries displayed oval depressions which were surrounded by a furrow and whose long diameter lay in the longitudinal axis of the vessel. A characteristic imprint is shown in Fig. 5B where the PMA and arterioli radiating to the sac are seen. Corresponding veins displayed a surface pattern with round depressions more randomly distributed (Fig. 5F). Differences in the surface structure on the casts corresponded to different endothelial outlines in the arteries and veins. Usually there was no characteristic surface pattern on vessels with a diameter of less than 6–8 μm.

The entire endolymphatic sac was surrounded by a dense vascular network which received branches from the PMA (Fig. 4A). The PMA entered the posterior cranial fossa close to the jugular vein, crossed the sigmoid sinus and broke up into a number of small branches. Some of them supplied the sac from the medial and distal directions. The PMA was accompanied by two meningeal veins which sometimes anastomosed with the sac vessels. The radiating arterioli which supplied the sac were about 20–50 μm in diameter and soon divided into capillaries (6–7 μm) over the sac. In 7 of the 35 animals a small artery arose from the posterior vestibular artery near the common crus and accompanied the vein of the vestibular aqueduct inside the bony aqueduct. It extended to the intermediate sac (Fig. 3B).

Anastomoses were observed between periaqueductal bone marrow sinusoids and perisaccular vessels. The perilabyrinthine bone marrow usually extended between the posterior semicircular canal and the vestibular aqueduct lying close to the anterior surface of the aqueduct. At SEM many small openings of bony canals were demonstrated on the anterior surface of the vestibular aqueduct, where blood vessels emerged (Fig. 4C), and microfil injection verified these direct communications between sac and bone marrow vessels inside the vestibular aqueduct (Fig. 3B).

The principal vascular supply and the distributory pattern of the endolymphatic sac vessels in the guinea pig are shown in Fig. 3C. The small arterial branch from the PMA can be seen. There is a dual arterial supply and centrally directed venous drainage towards the midportion of the sac. Its folded epithelium is invested in an extensive vascular network which by histology is also a prominent and characteristic feature of this area.

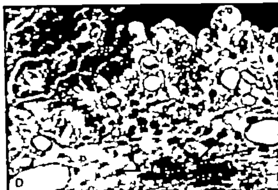
Histology

The relative vascular supply (vascular area/tissue length) of the different morphological regions of the endolymphatic sac was measured on serial sections and is plotted in a diagram in Fig. 6. The width of the perisaccular connective tissue in these regions is also shown. The most vascularized zone of the endolymphatic sac was its intermediate area around the operculum of the vestibular aqueduct. The proximal sac was less vascularized than the distal sac. The width of the perisaccular tissue layer was directly related to the degree of vascular supply, except for the region of the endolymphatic duct where the vascularization was poor in relation to the well developed surrounding loose tissue.

The endolymphatic duct was accompanied by the vein of the vestibular aqueduct which was sometimes split up into two or three venules. In addition, a small artery from the PVA sometimes coursed together with the vein. The proximal sac displayed capillaries and some thin walled venules which increased in number distally. In this region numerous small bony vascular channels contributed to the vascular supply.

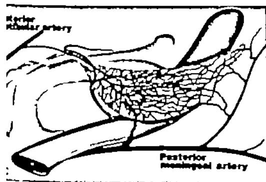
The intermediate sac exhibited a complex pattern of vascularization, being richly supplied with different kinds of vessels (Fig. 7A–D). In this area a large number of thin-walled irregular veins of varying size were predominant. Their irregular shape was clearly demonstrated by SEM of the vascular corrosion casts (Fig. 4D). Many small arteries and capillaries were also revealed by this method in this area of the endo[illegible]c sac (Fig. 5).











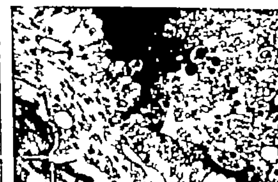


Fig 3A Guinea pig, right ear. Demonstration of the rich vascular plexus surrounding the endolymphatic sac by intravascular injection of silicone rubber. The bone tissue has been made transparent in methyl salicylate.

Fig 3B Guinea pig, left ear. Similar processing as in Fig 3A, showing the left vestibular aqueduct, which after clarification of the bone tissue is seen to house both an artery and vein.

Fig 3C Basic pattern of the vascular supply of the endolymphatic sac in the guinea pig. The sac overlies the sigmoid sinus and receives arterial tributaries medially and distally via branches from the posterior meningeal artery (*PMA*). A small artery reaches the proximal sac from the labyrinth and the posterior vestibular artery (*PVA*). It courses with the vein of the vestibular aqueduct parallel to the endolymphatic duct. Blood is drained in the central direction to the intermediate portion of the sac into the vein of the vestibular aqueduct down to the sigmoid sinus. The extensive vascular anastomoses in surrounding bony channels are not illus

Fig 3D Histological section showing part of the intermediate sac, its rugose epithelium, free cells and the numerous perisaccular vessels. The capillaries are lying close to the epithelial layer, while the thin-walled veins are situated peripherally. Guinea pig, paraphenylene diamine staining, phase contrast microscopy × 900.

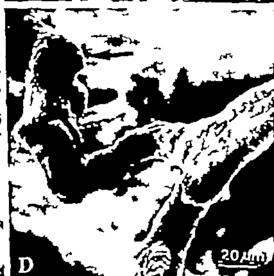
Fig 3E Histological section demonstrating the vein of the vestibular aqueduct (*right*) and the artery of the endolymphatic duct near the internal (vestibular) aperture of the vestibular aqueduct. Guinea pig, paraphenylene diamine staining, phase contrast microscopy × 250.

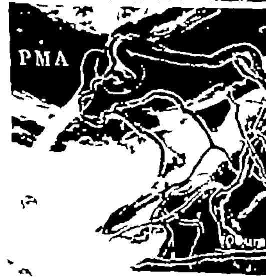
Fig 3F Histological section at the intermediate portion of the endolymphatic sac, showing opening of a vascular bony channel into the vestibular aqueduct. Serial sections demonstrate direct communication with the periaqueductal bone marrow sinusoids. Note the large number of subepithelially situated free cells as well as the numerous freely floating cells in the endolymphatic space. Guinea pig, toluidine staining × 500.

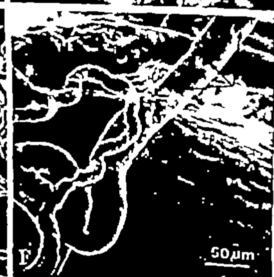

SS
A


20µm
B




20µm
D


PMA


50µm
F

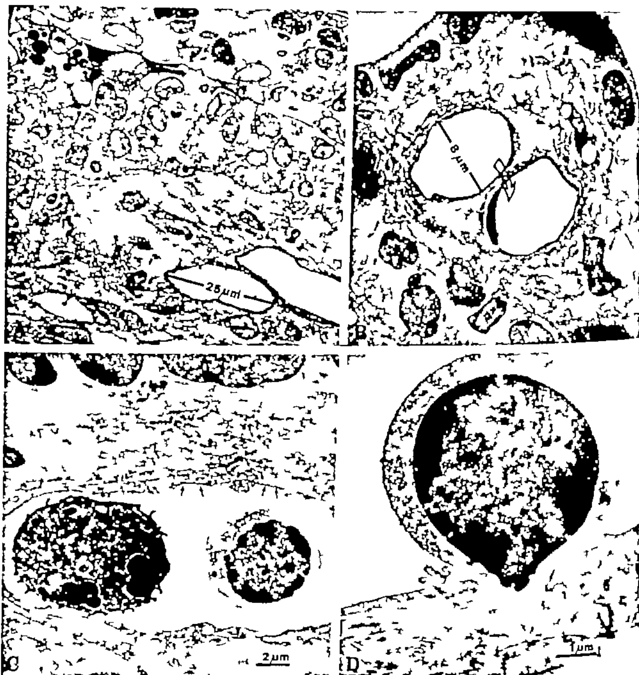


Fig 7A–D Transmission electron microscopy (TEM) of endolymphatic sac vessels Intermediate portion in the guinea pig (A) The intermediate sac epithelium is highly specialized and the loose perisaccular tissue contains numerous irregularly shaped thin-walled veins ×1400 (B) Vascularized epithelial buds with capillary loops in a connective tissue core were frequently observed ×3400 (C) A thin-walled perisaccular vessel The endothelial wall has numerous pores (*arrows*) covered by a thin membrane The vessel contains a polymorphonuclear leucocyte (*left*) and a lymphocyte (*right*) and a flocculent precipitate of plasma ×6000 (D) Lymphocyte diapedesis through the endothelial walls of the perisaccular veins was a common finding Serial sections demonstrate a passage through the endothelial cell into the perisaccular connective tissue rather than between cells ×15000

thelial walls (Fig 7*C D*) High resolution microscopy revealed lymphocyte and monocyte diapedesis (with a suggestion of pseudopods too) through the vascular wall and migration into the perisaccular tissue Lymphocytes were also found subepithelially between epithelial cells and in the endolymphatic space

Lymph vessels

Lymph vessels were scattered along the subepithelial tissue of the sac They were tenuous and irregular built up of a slender endothelial cell layer without a basal lamina, which made them easy to distinguish from veins which were often situated nearby They were most easily observed in the areolar tissue in the medial wall of the intermediate sac at the external aperture of the vestibular aqueduct. A collection of small lymph vessels (5–20 μm) formed a fine network in a spongy connective tissue layer containing long, stellate reticular cells and a few bundles of collagen fibres. The lumina of the lymph vessels often contained a proteinaceous precipitate and numerous white blood cells

DISCUSSION

Arterial blood reaches the endolymphatic sac from two directions The PMA which is a branch of the external carotid artery supplies the intermediate and distal sac with many (2–10) small radiating arteries In addition a small artery from the labyrinth supplies the endolymphatic duct and proximal sac This is a branch of the PVA which in turn arises from the labyrinthine artery The existence of this branch was verified histologically It was not invariably observed which may have been due to incomplete injection or to the fact that it is an anatomic variant which is not always present It seems to be of minor importance for the vascular supply to the sac as it is so finely calibred and furthermore despite poor filling of intralabyrinthine vessels in some cases the vessels of the endolymphatic sac were well outlined provided the PMA was adequately perfused Anson & Bast (1949) described a similar artery in man arising from the PMA and coursing with the endolymphatic duct for a short distance The branch described in this paper seems to run in the opposite direction but it cannot be ruled out that anastomoses exist between labyrinthine and meningeal arteries through the vestibular aqueduct, which could constitute an additional contribution to the arterial supply of the membranous labyrinth

The endolymphatic sac is a metabolically active structure In studies on the protein metabolism of the inner ear comparisons of the turnover rates of different structures of the inner ear have revealed that the synthetic activity of the intermediate sac is higher than that of the stria vascularis and the planum semilunatum but lower than that of the cochlear ganglionic cells (Koburg et al 1967) There is intense enzymic activity (Ishii et al 1966 Schätzle & Haubrich, 1966) and the cells of the mid-portion have many morphological characteristics indicating high cellular activity The sac capillaries have extremely thin walls and often exhibit endothelial pores indicative of fluid transport (Lundquist 1965) In the present investigation by using SEM of vascular corrosion casts numerous arteries which split up and supply the active epithelium around the endolymphatic sac were clearly demonstrated Also the extensive folds of epithelium in the sac contain cores of vascular connective tissue with capillaries arranged as loops as described earlier by Bast & Anson (1949) The complex plicate structure will therefore be surrounded by an extensive vascular system which seems consistent with its supposed function of reabsorption of water electrolytes and high molecular substances

The highly vascularized loosely textured connective tissue which is found exclusively around the proximal and intermediate portions of the endolymphatic sac is probably of great significance for the proper functioning of the sac Hallpike & Cairns (1938) noted that the area of the perisaccular connective tissue was diminished in patients with Meniere's disease and Zechner & Altmann (1969) described extensive fibrosis of the perisaccular framework in some cases particularly in patients with Meniere's disease and to a lesser extent in healed labyrinthitis The areas of areolar connective tissue were absent and the vascularization was definitely diminished Such changes

may be due to vascular disturbances primarily affecting the arterial sac supply or they may be a secondary phenomenon due to increased endolymphatic pressure causing compression of tissue against the bony aqueduct If an arterial supply is indispensable for normal functioning of the sac and a vascular organization similar to that in the guinea pig exists in man an operation such as ligation of the external carotid artery could jeopardize the nutritional supply of the sac and lead to functional disturbances However the small arterial branch from the labyrinth may establish an important anastomosis under such circumstances

This dual arterial supply and the fact that this portion of the labyrinth is nourished from a branch other than the vertebral arterial system (PMA) which in turn is a branch of the external carotid artery is interesting Portions of the external carotid artery develop early as a remnant of a small part of the first aortic arch It is therefore possible that the arterial anastomoses over the sac walls may constitute an embryonic remnant of the early connections between the external and internal arterial systems

The most prominent type of blood vessel encountered around the endolymphatic sac is the thin walled irregular sinusoid like vessel This type of vessel is unique to the inner ear and they have been classified as sinusoids (Lundquist et al 1971) However they seem to belong to the venous side of the vascular tree anastomosing freely with the bone marrow sinusoids and allowing the passage of fluid metabolites and free cells across its endothelial wall According to the original description of sinusoids the term should be restricted to those vessels in the liver spleen and endocrine organs with a specific histiogenesis The sac vessels are probably structurally differentiated venules and veins adapted to fit a specific function exerted by the endolymphatic sac tissue

It is inevitable to discuss the function of the endolymphatic sac vessels in relation to the origin of the free luminal cells of the sac. A striking property of the endolymphatic sac is its great ability to phagocytose exerted both by epithelial cells and free luminal cells (Guild 1927 Andersen 1948 Lundquist, 1965). In contrast to other areas of the membranous labyrinth the lumina of the proximal and intermediate portions of the sac contain numerous white blood cells and histiocytes whose origin and fate are unknown Histological serial sections in this investigation showed a simultaneous occurrence of luminal cells, increased endolymph stainability and increased vascularity of the proximal perisaccular tissue. The freely floating cells of the sac probably derive from its perisaccular tissue and sac epithelium rather than from inside the labyrinth (Schätzle & Haubrich 1966) The rich vascular network surrounding the sac, comprising both the perisaccular vessels and bone marrow sinusoids seems able to provide the endolymphatic space with a multitude of these free cells

This may imply that the perilabyrinthine especially the periaqueductal bone marrow space which is connected to the vestibular aqueduct is important for the proper functioning of the endolymphatic sac. This observation is clinically relevant since it has been found in a tomographic study that in most patients with Meniere s disease the periaqueductal pneumatization of bone marrow space of the pyramid is either absent or sparse as compared with a group of healthy individuals and that this absence or sparsity reduces the tomographic visibility of the vestibular aqueduct (Stahle & Wilbrand 1974)

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die Gefäßanatomie des Saccus endolymphaticus beim Meerschw[illegible] von Sihk[illegible]

resin wurde angewandt, um Korrosionsgefäßabgüsse für Raster-Elektronenmikroskopie zu bekommen, wodurch eine genauere Unterscheidung zwischen Venen und Arterien erzielt wurde. Das ausgedehnte Gefäßsystem um den Saccus herum enthält sowohl Arterien als auch Venen und Lymphgefäße. Die Gefäßversorgung kommt hauptsächlich von der meningea posterior der hinteren Schädelgrube. Eine kleine Arterie erreicht den Saccus auch von der cerebellaris posterior im Labyrinth (bei 7 von 35 untersuchten Tieren). Sie verläuft gemeinsam mit der Vene des Aquaeductus vestibuli entlang der Wandung des Ductus endolymphaticus. Der Blutstrom geht über den intermediären Abschnitt des Saccus und umgibt diesen mit einem dichten Netz von Kapillaren, Venülen, Venen und einigen kleinen Arterien. Einzelne Venenstämme aus der Saccuswandung vereinigen sich mit der Vene des Aquaeductus vestibuli, welche das Vestibularblut in den Sinus sigmoideus drainiert. Mit der Raster-Elektronenmikroskopie konnten auch zahlreiche Gefäßanastomosen mit benachbarten Knochenmarksräumen über knöcherne Gefäßkanälchen dargestellt werden. Diese Verbindungen tragen wahrscheinlich zur Gefäßversorgung des Saccus endolymphaticus bei.

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ZUSAMMENFASSUNG

Die Gefäßanatomie des Saccus endolymphaticus beim Meerschweinchen wurde mit intravaskulärer Injektion von Silikongummi (Microfil) untersucht Methacrylat

satisfactory results It gives the most even contrast without penetrating the vessel wall

Soft-surface specimen technique and contrast injection

A modification of the contrast injection method is its use in conjunction with techniques for evaluating the sensorineural epithelium After contrast injection, preparations are decalcified stained with osmium and all areas of the cochlea are removed for viewing with phase-contrast microscopy as surface preparations The advantage of decalcified over undecalcified material is that all parts of the cochlea as well as the spiral ganglion modiolus and acoustic nerve become accessible for observation. The technique itself is advantageous for studying the inner ear vessels in relation to the lightly-stained sensorineuro-epithelium Areas with pronounced sensory cell loss can be carefully evaluated with respect to the vasculature This method is also suitable for studies of mechanical lesions and in addition can be used for studies of congenital disorders where both sensorineuro-epithelium and vasculature might be expected to be affected

Surface specimen methods in decalcified preparations without vascular perfusion using the double-blind assessment technique

While the contrast injection and surface specimen techniques proved useful for observing areas of gross vascular pathology more detailed information on the status of individual vessels was either missing or impossible to evaluate By perfusing the vessels with contrast injections we gained visibility but lost valuable information about the intravascular contents We needed a technique which allowed us to evaluate more discrete intra- and peri-vascular changes without sacrificing visibility After much trial and error we are at present using a surface specimen method with decalcified preparations in which vascular perfusion i omitted This method allows us to study intravascular cellular elements as well as the vascular lumen and perivascular elements. As in the previous technique the vasculature can be studied in relation to the sensorineurol epithelium in all parts of the cochlea. Again decalcification permits all areas of the cochlea to be examined. We have not yet been able to determine the extent of artifactual change in the sensorineuro-epithelium or vasculature after careful decalcification with EDTA or Versene At least with magnifications up to 500× gross artifactual changes have not interfered with our measurements. Our present method will be presented in greater detail

Preparation and Dissection

Animals are sacrificed without anesthesia by decapitation with sharp scissors or a guillotine The temporal bones are immediately removed by a dorsal approach and the bullae opened Under low-power magnification the stapes is removed and a small opening is carefully made in the cochlear apex The cochlea is then fixed with 2% glutaraldehyde which is slowly injected through the oval window and apical openings After 24 hours in the fixative the cochlea is decalcified in 8% EDTA which is changed daily until the decalcification process is complete The time necessary for decalcification can vary according to the experimental animal used and according to the experimental treatment undergone Cochlear implants for example often lead to chronic otitis and new bony growth in the bulla and thus specimens require a longer time to decalcify Generally at least in the guinea pig decalcification does not usually exceed 7 days A mid or paramodiolar apico-basal section of the cochlea is then made using a single-edged razor blade On the cut surface all structures are examined for gross changes such as the presence of debris—possibly from degenerating tissue fibrous growth around an electrode or other type of implant A rough appraisal of the vasculature is also made being especially attentive for intracochlear hemorrhage at the

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METHODOLOGICAL ASPECTS OF SOME INNER EAR VASCULAR TECHNIQUES

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Abstract Modifications of preparation technique assessing cochlear vasculature together with suggestions regarding their applicability to various types of research projects are presented Detailed information is given regarding the soft-surface dissection and preparation technique along with selected parameters for evaluating the cochlear vasculature Some basic requirements for any histological technique studying the inner ear blood supply are suggested

The inner ear vasculature has been a subject of interest for many researchers Most early work was aimed at determining and charting the cochlear vascular anatomy This was usually accomplished by using a vascular contrast perfusion technique Our own research also began with such a technique As our scientific questions evolved beyond that of basic anatomy it became necessary to modify and improve our histological technique It is our intention to present some of these modifications of the basic contrast injection technique with suggestions regarding their applicability to various types of research projects

Different Types of Methods for Studying Cochlear Vasculature (Fig 1)

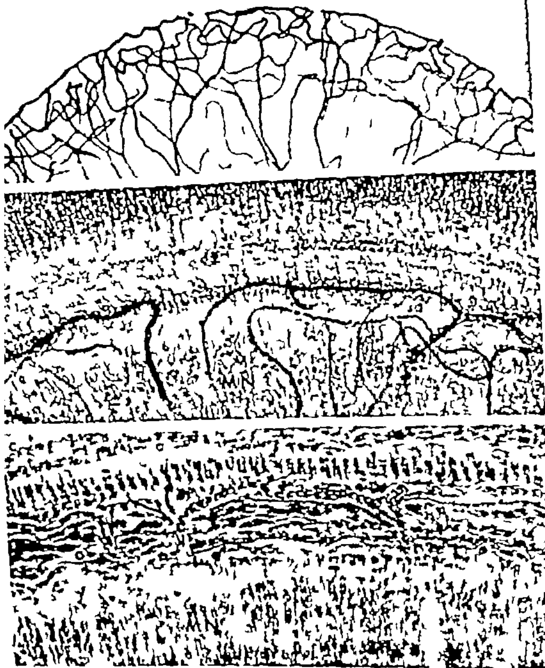
(1) Contrast injection technique and light microscopy (Eichler 1892 Siebenmann 1894 Nabeya 1923 Smith 1951 Axelsson 1968 1972)

(2) A combination of contrast injection and surface specimen methods in decalcified preparations using phase contrast microscopy (Axelsson et al 1974 1975)

(3) Surface specimen methods in decalcified preparations without vascular perfusion using phase contrast microscopy and double-blind assessment (Axelsson & Vertes, 1977 Vertes et al in press)

Contrast injection techniques

The cochlear vessels have been perfused with solutions such as India ink Berlin blue and more recently Methacrylate resin (Hodde et al 1977) The basic idea is to wash out the intravascular elements and fill the vessels, even the most delicate capillaries with a contrast medium Using the conventional contrast injection method the vascular anatomy is observed in decalcified specimens with light microscopy External wall and spiral lamina preparations are usually mounted on glass slides in glycerin When resin is used all cochlear tissues are macerated and only the casts of the vessels remain These are then visualized using scanning electron microscopy The advantage of the contrast injection method is that it gives an excellent view of all cochlear vessels, and consequently is good for studies of normal anatomy It can also be adopted to study gross injury healing and possible regeneration of the cochlear vasculature such as occur after mechanical lesions (Axelsson & Hallén 1973) Since this method involves removal of the blood elements it is not suitable for examining more minute influences on the cochlear vasculature One disadvantage of this technique is that it is often difficult to get an even and complete contrast injection Having evaluated many different contrast media we have found Berlin blue to give the most



F[illegible] 1 Three methods for studying cochlear vasculature. [illegible]) Contrast injection, light microscopy. This method is appropriate for studies of vascular anatomy and major vascular pathology at low magnifications. (*b*) Contrast injection, osmium staining, phase-contrast microscopy. This method can be used for viewing vessels in relation to sensorineuro-epithelium. MN, myelinated nerve fibres. ( ) No contrast injection, osmium staining, phase-contrast microscopy. This method shows detailed intra- and perivascular histology in addition to the sensorineuro-epithelium. MN, myelinated nerve fibres.

Table I *Regularly occurring vessels of the cochlea*

EXTERNAL WALL
Scala Vestibuli
Radiating arterioles
Collecting venules
The vessel at the vestibular membrane
Scala Media
Arteriovenous anastomoses
Stria vascularis
The vessel of the spiral prominence
Scala Tympani
The venules at the basilar membrane
Collecting venules
SPIRAL LAMINA
Radiating arterioles & Collecting venules
The limbus vessels
The vessel of the basilar membrane
The vessel of the tympanic lip

round window and at the cochlear acqueduct The condition and position of the vestibular and basilar membranes are also assessed at this time The cochlea is then counterstained in 0 5% osmic acid for up to 10 minutes dehydrated in an alcohol series of increasing concentration and stored in glycerin Further dissection consists of removing surface speci mens in successive half turns first by making cuts through the modiolus as close as possible to the organ of Corti After this the modiolus is gripped firmly with a pair of forceps and the specimen is eased away from the mem branous external wall The basilar membrane usually separates quite easily from the spiral ligament at the Claudius cell layer In the basal turn however where dissection is most difficult fine glass knives may assist in freeing

Table II *Evaluation of red blood corpuscles*

Density – frequency and spacing of red blood corpuscles in vessel lumen
Columns – number of rows of red blood corpuscles in vessel lumen
Aggregation & plasm gaps – spaces between collec tions of red blood corpuscles
Orientation – manner and plane in which red blood corpuscles are located in vessel lumen

Table III *Evaluation of perivascular cells*

Perivascular cell frequency – occurrence of endothelial cell nuclei and/or pericyte
Perivascular cell size – diameter and length of endothelial cell nuclei and/or pericyte
Perivascular cell compressing lumen frequency – occurrence of narrowed vessel lumen caused by endothelial cell nuclei and/or pericyte

the specimen from the external wall The vestibular and tectorial membranes are removed from those specimens in which cell counts will be made The entire spiral ligament including the radiating arterioles of the scala vestibuli the stria vascularis and the collecting venules of the scala tympani is then removed This is done using a type of scoop which is easily inserted between the membranous and bony (but decalcified) external walls The modiolar and external wall specimens are placed on a glass slide for further examination using phase or interference contrast microscopy

Evaluation of the Vasculature

All examinations are done double-blind, that is without knowing whether the specimen belongs to the control or experimental group The method involves assigning a subjective value to each of the adopted parameters based on observations of vessels made within the whole microscopic field Three major aspects of the vasculature—red blood corpuscles, perivascular cell and the vessel lumen—are investigated in all the regularly occurring cochlear vessels (See Table I) Specific parameters and their definitions are shown in cochlear vessels Table I Specific parameters and their definitions are shown in Tables II, III and IV examples are given in Figs 1 and 2

Table IV *Evaluation of vessel lumen*

Lumen irregularity—local narrowing and widening of vessel lumen
Vessel lumen diameter – width of vessel lumen

ble V *Additional vascular parameters*

vascular spac
vels lacking blood corpuscles
le blood corpuscle
bubbles in vessel bound clear spaces within vessel
men
cells osmiophilic materials which surround ves-
els and are larger than perivascular cells
ocytes – prolonged or amebiform pigment cells
then having 3 arms or prolongations
ule pigment formed of fine granulations which
pear free in the cytoplasm
ment clump clusters or collections of granula
ole in strial surface structure – bound, clear space
ithin the strial bed
es between cells spaces occurring between strial
surface cells
ocyte between VSBM and VSTL – cells of loose
connective tissue located between the spiral vessels of
he spiral lamina
capillary sphincter – narrowing of vessel usually
both sides simultaneously presumably by peri-
vascular elements that surround it

oted in all or selected cochlear vessels Pigmentary cells are defined according to Savin (1965) All values are recorded on a work sheet which can be directly programmed for computer analysis

DISCUSSION

The following criteria are thought to be important requirements for the study of the cochlear blood supply 1) It must be possible to remove the entire vasculature with a minimum of tissue loss 2) In order to be able to evaluate localized changes all vessels must be visible throughout the cochlea 3) Elements inside and outside the vessels should be seen. 4) The structural relationships between and among vessels should be obvious and they should be as similar as possible to the in-vivo condition 5) The structural relationships between vessels and other supporting sensory and nerve cells should be obvious and they should be as similar as possible to the *in-vivo* condition 6) The method should be compatible with other techniques by which hair cell or nerve damage is estimated 7) The method should be simple reproducible and rapid

After having evaluated the cochlear vasculature for a number of years using a variety of histological techniques we believe that our present method satisfies most of these requirements quite well The double-blind assessment technique is thought to be especially important in eliminating or at least diminishing experimental bias resulting from expected results

We would like to point out that our method of evaluating the vasculature is quite flexible Since we still do not know for certain which parameters are significant in the evaluation of vascular pathology we have sought to develop a system which would allow the addition or deletion of factors without necessitating a change in the entire system Thus future simplification of the system—by deleting some parameters—is possible in the event that those parameters do not distinguish between control and experimental animals.

With the exception of anatomical studies the cochlear vasculature is very rarely described in its entirety Thus while there is a wealth of data on the effect of various stressors on individual cochlear vessels both at the light and electron microscopic levels, our knowledge is limited as to how the cochlear vascular system as a whole is affected Just as accurate estimates of hair cell damage can only be obtained by evaluating the entire cochlea, so does it seem equally important to evaluate the vasculature in all areas of the cochlea. The appearance of any one cochlear vessel at any instant is clearly dependent on the interaction of all the vessels in the vascular supplying and draining the cochlea. We believe our technique can contribute important information about this cochlear vascular system

ZUSAMMENFASSUNG

Vollständige der Methoden für die Darstellung der Cochleagefäße und deren Verwendungsmöglichkeiten bei verschiedenen Untersuchungen werden beschrieben. Genaue Information und über Dissektion und Präparation mit der "weichen-Flächen" (soft-surface) Technik

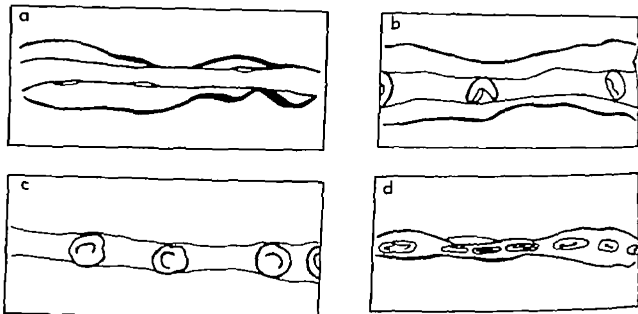


Fig 2 Schematic representation of cochlear vessels (*a*) *Density* (0): no visible cellular components *Vessels lacking blood corpuscles* present *Perivascular space* present *Perivascula cell diameter* slim *Perivascul r cell length* short. (*b*) *Density* (1): spaces greater than a length of 5 red blood cells between individual red blood cells *Orientation* OK oblique *P rivascular space* present (*c*) *Density* ( ): approximately equal spaces w th lengths from 1–5 red blood cells between individual red blood cells *Orientation* OK transverse (*d*) *Density* (3) red blood cells lying close to each other but not touching *Orientation* pressed longitudinal and OK longitudinal *Lumen irregularity* present; *Perivascular cell diameter* slim *Perivascular cell length*, extended. *Perivascula cell compressing lumen* present

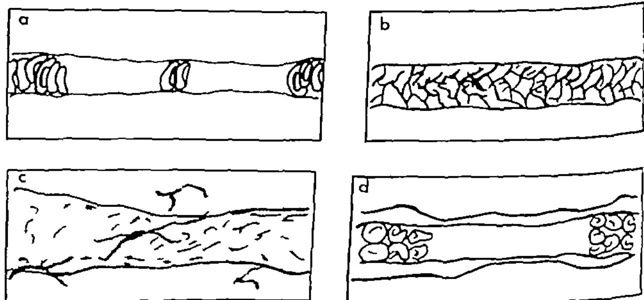


Fig 3 Schematic representation of cochlear vessel (*a*) *Density* (4) touching and o erlapping red blood cell which still appear in disc formation *Ori tation* OK transverse *Plasma gap* present (*b*) *Density* (5): ove lapping red blood cells no longer in disc projections in which cell boundaries can still be d stinguished *Orientation* pressed oblique *Number of rows* –3 (*c*) *Density* (6) o erlapping amorphou mass of red blood cells i which cell boundaries cannot be seen *Vessel lumen d amete* d lated *Melanocyte* present (*d*) *Density* (4): touching and overlapping red blood cell which till appear i disc formations *Orientation* OK transverse *Plasma gaps* present *Number f row* *Perivascular space* pres

HYPERBARIC OXYGEN AND STELLATE GANGLION BLOCKS FOR IDIOPATHIC SUDDEN HEARING LOSS

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Abstract. Ninety-one patients suffering from idiopathic sudden hearing loss are presented. Twenty-two patients were given medical treatment (vasodilators, steroid hormones and vitamins) alone (group 1). Forty-nine patients were treated with stellate ganglion block (SGB) plus oxygen hyperbaric therapy (OHP) (group 2) and 20 patients were treated with SGB plus OHP along with medical treatment (group 3). The SGB plus OHP treated patients were given bupivacaine, which induced Moore's anterior approach of SGB and then exposed to oxygen at pressure of 2.4 ATA for 90 min. In group 1 69% of the patients treated within one week after onset exhibited over 10 dB pure tone average improvement, with only 33% patients treated one to two weeks after onset experiencing over 10 dB. However 74% of the patients in group 2 and 100% of the patients in group 3 who were treated within two weeks after onset exhibited over 10 dB improvement. More significantly of the patients which experienced complete loss of hearing, 83% in group 2 and 100% in group 3 exhibited over 10 dB improvement, compared to only 33% in group 1. Moreover 8 (40%) patients in group 3 recovered to within 20 dB of their normal hearing levels. In group 2, 17 patients were treated two to six weeks after onset and 12 (71%) patients had over 10 dB improvement. SGB plus OHP therapy was shown effective in the treatment of sudden idiopathic hearing loss even when patients were treated more than two weeks after onset.

The cause of sudden idiopathic deafness is thought to be vascular or viral in origin resulting in a reduced blood supply to cochlea. However there does not seem to be any agreement among otolaryngologists regarding treatment for sudden hearing loss. Many researchers have reported varying degrees of success of treatment through the incorporation of a number of compounds such as vasodilators, anticoagulants, vitamins, steroid hormones and tranquilizers.

Schubert (1949) and Hilger (1950) reported the results of treatment by blocking the stellate ganglion (SGB) in the therapy for sudden hearing loss. Haug et al. (1976) reporting recently on a total of 56 patients indicated symptomatic improvement in 70% of the cases in which SGB was performed compared to only 20% of the non-SGB treated patients. Significantly 91% of the patients who *improved* were treated within the first two weeks following the onset of symptoms. The authors therefore stressed the importance of early *treatment*.

Appaix et al. (1970) and Lamm (1971) reported an initial experience with the use of hyperbaric oxygen therapy (OHP) in the treatment of inner ear disorders. Yanagita (1974) and Yanagita et al. (1976) also reported better results in patients treated with OHP than in patients treated with vasodilators, steroid hormones, vitamins or SGB.

Conversely, constriction of cerebral blood vessels upon induction of oxygen was reported by Lambertsen et al. (1953). Murata et al. (1974) reported that depressed cochlear microphonics due to hyperbaric oxygen induction was allayed with the resection of the superior cervical ganglion in guinea pigs.

This report is a presentation of the results from a series of studies on OHP combined with SGB in order to inhibit constriction of blood vessels due to induction of hyperbaric oxygen.

MATERIAL AND METHODS

The typical patient is one who suddenly become hard of hearing or deaf in one ear for no apparent reason. Associated symptoms are

gegeben Zweckmäßige Parametern fur die Beurteilung werden vorgeschlagen Die Anforderungen die grund sätzlich bei jeder histologischen Technik zum Studium der Gefäße des Innenohres gestellt werden müssen werden vorgeschlagen

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ble II *Extent of loss and pu e tone improvement*

es treated within tw weeks after onset

ent of los erage 500– 000 Hz)	N	Age (mean)[a]	Interval before treatment (days) (mean)[a]	Pure tone improvement (dB) (mean)[a]	Improved cases	Recovered cases[c]
oup 1 (medical eatment)						
0–49 dB		36	5	36	2 (100%)	2
0–79 dB	8	42	6	4	6 (75%)	1
0 dB	1	40±6	8±1	11±4[a]	4 (33%)[d]	0
oup (SGB OHP)						
0–49 dB		33	9	21	2 (100%)	1
0–79 dB	5	44	7	22	2 (40%)	1
0 dB	12	40±4	9±1	38±8[b]	10 (83%)[e]	1
oup 3 (SGB OHP medical treatment)						
0–49 dB	3	31	7	26	3 (100%)	2
0–79 dB	6	39	7	37	6 (100%)	4
0 dB	11	36±4	9±1	42+6[c]	11 (100%)[f]	2

The data of total hearing los type patients (80 dB) alone were analysed statistically

The patients achieved over 10 dB pure tone improvement (erage. 250–4 000 Hz)

The patients stabilized within 20 dB (500– 000 Hz) hearing loss

atistical analysis b. a. $P<0.01$ >d $P<0.05$ f>d. $P<0.01$

nd OHP within two weeks after onset of hearing loss Patients treated after two weeks following the onset of symptoms were treated ith SGB and OHP alone as medical treatment has little effect on patients treated two eeks or more following the onset of symptoms Also 15 patients treated with SGB and OHP two weeks following the onset of symptoms had already been treated with medical drugs but exhibited no significant improvement Of course hearing level recovery rates were compared by examination of the audiogram for the first day and the last SGB and OHP therapy day

Hearing was considered to have impro ed when recovery of a pure tone average (250–4000 Hz) of over 10 dB was attained In two cases the improvement rate fluctuated following the development of sudden loss of hearing and they are excluded from the final results. Patients whose audiogram stabilized within 20 dB (500– 000 Hz) of hearing loss were considered to have recovered normal hearing ability

Student s t test for paired or unpaired comparisons was used for statistical analysis with a P value of less than 0 05 considered to represent a statistically significant change

RESULTS

Table I shows the per cent of improved patients according to the method of therapy and the number of days treatment was delayed following the onset of symptoms Sixty-two (68%) patients achieved substantial hearing improvement. Forty-six improved cases were treated within two weeks after the onset of symptoms Every patient in group 3 improved In groups 1 and 2 the rate of improvement in patients treated within one week following the onset of symptoms was similar However in group 1 patients treated later than one week following the onset did not achieve over 30 dB pure tone improvement and only 3 (33%) patients were actually found to have improved. Thirty patients received SGB plus OHP therapy following more than two weeks after onset

Table I *Pure tone average improvement in patients treated with stellate ganglion blocks (SGB) and hyperbaric oxygen therapy (OHP) as compared with those in whom medical treatment was used*

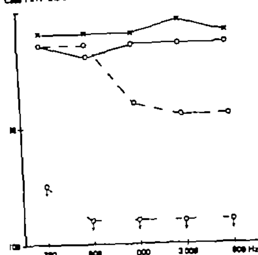
Interval before treatment	No	Pure tone improvement (average 250–4 000 Hz) Over 30 dB	29–20 dB	19–10 dB	9–0 dB	Improved Cases*
Group 1 (medical treatment)						
Within one week	13	5			4	9 (69)
Within two weeks	9	0[d]	2	1	6	3 (33)[b]
Group (SGB + OHP)						
Within one week	6	3	1	1	1	5 (83%)
Within two weeks	13	5[e]	?	2	4	9 (69%)[c]
Within four weeks	9		3	2		7 (78%)
Within six weeks	8	0	1	4	3	5 (63)
After six weeks	13	1	1	2	9	4 (31%)
Group 3 (SGB + OHP + medical treatment)						
Within one week	11	7	2		0	11 (100)
Within two weeks	9	4	2	3	0	9 (100%)[f]
Total	91	27	16	19	29	6_ (68%)

The patients achieved over 10 dB pure tone improvement (average 250–4 000 Hz) Statistical analysis c>a $P<0\ 0$ b>a n.s e>d $P<0\ 01$ f>d $P<0\ 01$

tinnitus quality distortion of sound recruitment intolerance to loud sounds and at times fullness in the ear and vertigo also without apparent cause Conditions that are known to produce abrupt usually unilateral sensorineural losses such as mumps measles acoustic tumors ear surgery and ototoxic drugs were excluded from this case report.

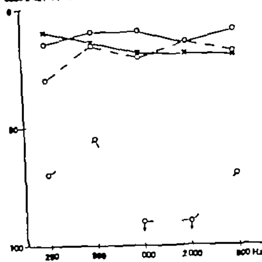
SGB and OHP therapy were employed for 69 patients suffering from sudden sensorineural hearing loss in this series The control group (22 patients) was treated with dexamethasone vitamin B kallidin (kallikrein Bayer) and nucleoside mixed with hydroxy methyl-propanol All patients were informed of the nature of treatment in detail and written consent obtained prior to initiation of therapy The average age of the patients was 39 years Forty-eight (53%) were men and 43 (47%) were women and all patients were suffering from sudden unilateral hearing loss

The SGB procedure incorporated was Moor s (1965) anterior approach The technique consists of an injection of 6 to 8 ml 0 25% bupivacaine and observation of the patients until the onset of Horner syndrome. Thirty min following the injection patients were exposed to oxygen pressures of 2.4 ATA for 90 min Each patient was routinely treated 20 times during a period of 4 weeks Once a week patients were reevaluated audiologically Patients were divided into three recovery groups depending upon the degree of improvement Group A (8 patients) consisted of patients which recovered completely within 20 treatment periods Patients exhibiting consistent improvement rates but not yet fully recovered following 20 treatment periods were placed in group B (22 patients) Patients whose rate of recovery had stabilized within 20 treatment periods and showed no further signs of possible improvement were placed in group C (39 patients) Patients in group B continued to receive treatment for up to one to three weeks as their recovery rates after 20 periods indicated that significant recovery was possible with further treatment Of the 69 patients 70 were treated with drugs combined with SGB



onset of hearing disturbance 12 1 1976

- 2 13 976 before treatment
- 12 25 1976 after ten treatment periods
- 8 1976 after twenty treatment periods
- 1 19 1976 left ear



onset of hearing disturbance 7 1977

- 7.28.1977 before treatment
- 8 4 1977 after seven treatment periods
- 8 10 1977 after eleven treatment periods
- 10 1977 left ear

g. 1 Pure tone audiometric test results obtained prior and after SGB and OHP therapy

SGB Hoarseness and light brachial plexus paralysis due to SGB were sometimes observed but disappeared within a few hours

CASE REPORTS

The following cases are presented in more detail to demonstrate certain notable aspects of the history and/or the effect of treatment

The two cases shown in Fig. 1 were treated with SGB and OHP alone Case 1 is a total hearing loss type but the patient recovered normal hearing level after 20 treatment periods with SGB and OHP Case 2 was a middle tone deafness type and recovered completely with only 11 treatment periods

Two young cases in Fig. were treated with vasodilators steroid hormones and vitamins for two months Hearing in the left ear of case 4 was recovered with medical treatment but the audiogram of the right ear did not change In both cases the effect of the treatment with SGB and OHP therapy was not significant for the first few weeks but pure tone hearing level increased remarkably during the following 10 treatment periods.

DISCUSSION

The major difficulty in evaluating the results of treatment involves the lack of a pertinent control series and the fact that spontaneous recovery has been reported by many otologists (Simmons 1973) For this reason there is no general agreement on the accepted form of treatment or whether any treatment is in fact worthwhile in the majority of cases. Simmons (1973) suspected that the incidence of sudden hearing loss is probably much more frequent than is documented Spontaneous recovery

Table III *Age at onset of symptoms*

Cases treated with stellate ganglion blocks and hyperbaric oxygen therapy

Age	No	Pure tone improvement[a] (average 250–4 000 Hz)	Improved cases[b]	Recovered cases[c]
Under 30	19	31±5 dB[a]	17 (89 %)[e]	6 (32 %)[p]
30–39	14	3 ±7 dB[b]	11 (79 %)[f]	5 (36 %)[q]
40–49	17	20±4 dB[c]	12 (71 %)[g]	1 (6 %)[r]
Over 50	19	16±4 dB[d]	10 (53 %)[h]	1 (5 %)[s]
Total	69	25±3 dB	50 (72 %)	13 (19 %)

Mean ± S E M

Statistical analysis a>d $P<0\ 05$ b>d $P<0\ 01$ e>h $P<0\ 01$ f>h $P<0\ 05$ p>s: $P<0\ 02$, q>s, $P<0\ 02$

The patients achieved over 10 dB pure tone improvement (average 250–4 000 Hz)

The patients stabilized within 20 dB (500–2 000 Hz) hearing loss

of symptoms Of these sixteen (53 %) patients had over 10 dB improvement and 3 patients achieved over 30 dB Two patients stabilized within 20 dB (500–2 000 Hz) of hearing loss (one audiogram is shown in Fig 2 [case 4])

Table II shows the effect of SGB plus OHP or medical treatment in patients suffering from sudden hearing loss in cases treated within two weeks of the onset of symptoms Twenty two patients were treated with drugs alone (group 1) Nineteen patients were treated with SGB plus OHP (group 2) and 20 patients were treated with SGB OHP and medical treatment (group 3) The patients were divided into three groups relative to the degree of hearing loss In all three groups the data on total hearing loss patients (80 dB+) were analysed statistically In group 2 83 % of the patients improved and 100 % of the patients improved in group 3 compared to only 33 % in group 1 The average pure tone improvement was also significantly higher in groups 2 and 3 Recovery in 14 patients (3 patients each in groups 1 and 2 8 patients in group 3) was accompanied by stabilization at a point within 20 dB (500–2 000 Hz) of the normal range

Table III shows the age at the onset of symptoms There was a fairly even distribution of patients in the 12–29 30–39 40–49 and 50 age brackets The rate of improvement in the overfifty bracket was significantly low On the other hand in the under 30 age bracket, 17 patients (89 %) exhibited pure tone improvement of over 10 dB and six patients recovered within 20 dB hearing loss Eleven of the 13 patients who recovered were under 40 years of age

Every fourth improved patient (Table I, group 2) treated after six weeks following the onset of symptoms was under 40 years old.

Vertigo was present in 38 (42 %) patients. Twenty-one of the 27 patients which exhibited over 30 dB pure tone improvement (Table II) and 11 of the 14 patients which recovered completely (Table II) did not have vertigo Statistical comparison of the rate of improvement between these two groups cannot be carried out as the large number of patients who had no vertigo received therapy at an early stage

Complications as a result of SGB and OHP therapy were very rare One patient suffered convulsions for a brief time in the hyperbaric oxygen chamber but exhibited no further complications after the convulsions had ceased and was again subjected to SGB and OHP therapy two days later Three patients were unable to undergo OHP therapy because of otalgia during atmospheric pressure changes. They were treated with medical treatment and

40% chance that normal hearing level will be regained with SGB and OHP combined with medical treatment within two weeks after onset (Table I group 3) Moreover 12 (71%) patients treated later than two to six weeks following the onset achieved over 10 dB pure tone improvement (Table I group 2) However the delay in time from the onset of symptoms to initiation of our therapy seems to be an important factor and in this respect is similar to the results forwarded in earlier reports.

Murata et al (1974) have shown that depressed cochlear microphonics due to induction of hyperbaric oxygen was allayed by the resection of the cervical ganglion Medical drugs such as vasodilators, vitamins, steroid hormones and the like also seem to be effective in increasing cochlear blood flow (Suga & Snow 1969) We believe that SGB and OHP therapy is effective in the treatment of sudden idiopathic hearing loss However when this therapy is not combined with medical treatment, the results are less significant.

In the light of data contained in reports cited here and the results presented in this paper it would seem appropriate to further expand the application of SGB plus OHP accompanied by medical treatment to patients who receive their initial treatment more than two weeks following the onset of hearing loss

ZUSAMMENFASSUNG

Vorgestellt werden 91 Patienten die an plötzlichem, idiopathischem Gehörverlust leiden 22 Patienten (Gruppe 1) wurden nur medikamentös behandelt (mit Vasodilatoren-Mittel, Steroid-Hormonen und Vitaminen) 49 Patienten (Gruppe 2) wurden mit SGB behandelt und erhielten zusätzlich eine hyperbare Sauerstofftherapie (OHP) und 20 Patienten (Gruppe 3) erhielten SGB plus OHP plus medikamentöse Behandlung Die Patienten, die mit SGB und OHP behandelt wurden (Gruppen … 3) erhielten Bupivacaine das die vorhergehende SGB-Behandlungsmethode von Moor einleitete und dann wurde ihnen 90 Minuten lang Sauerstoff unter dem Druck von 2.4 ATA zugeführt In Gruppe 1 wurde bei 69% der Patienten, die innerhalb einer Woche nach Ausbruch der Krankheit behandelt wurden eine durchschnittliche Besserung der reinen Tonaufnahmefähigkeit von über 10 dB festgestellt wobei nur 33% der Patienten, die eine bis zwei Wochen nach Ausbruch der Krankheit behandelt wurden eine Besserung von über 10 dB erlebten. Indessen zeigten 74% der Gruppe 2 und 100% der Gruppe 3 die innerhalb von zwei Wochen nach Krankheitsausbruch behandelt wurden, über 10 dB Besserung. Noch bedeutender war es, daß von den Patienten mit totalem Gehörverlust 83% aus Gruppe 2 und 100% aus Gruppe 3 eine Besserung von über 10 dB zeigten verglichen mit nur 33% aus Gruppe 1 Darüber hinaus erlangten 8 (40%) Patienten aus Gruppe 3 bis zu 20 dB des normalen Niveaus ihres Hörvermögens zurück. In Gruppe wurden 17 Patienten zwei bis sechs Wochen nach Ausbruch der Krankheit behandelt und 12 (71%) von ihnen hatten eine Steigerung von über 10 dB SGB mit zusätzlicher OHP-Behandlung erwies sich bei der Behandlung von plötzlichem idiopathischem Gehörverlust als wirkungsvoll, selbst wenn die Patienten später als 2 Wochen nach Krankheitsausbruch behandelt wurden.

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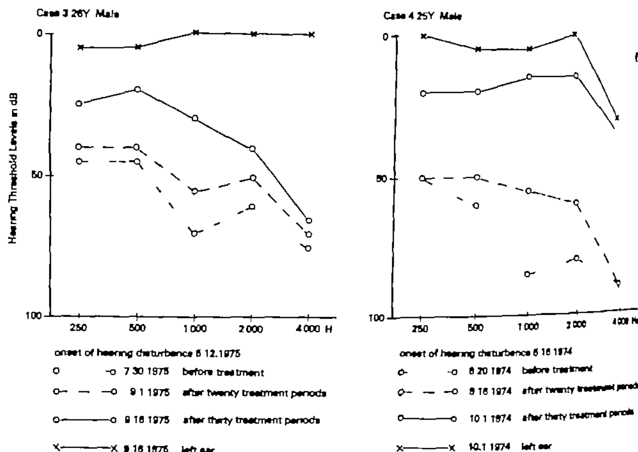


Fig 2 Pure tone audiometric test results obtained prior to and after SGB and OHP therapy

does occur sometimes within a few hours after the onset When that interval is three days or less 68% of the patients can be expected to recover completely He suggested that the recovery rate for patients treated within two weeks after onset is independent of the type of treatment Shaia & Sheehy (1975) reviewed 1200 cases and reported the effects of medical treatment with vasodilators for 380 patients Of the treated group 40% exhibited significant hearing improvement. There was a direct relationship between the time of onset of treatment and the number of patients who showed improvement However the rate of recovery is very low among the patients who experienced complete loss of hearing (Sheehy 1960) It is most important to compare the recovery rate of the patients which experienced total hearing loss when the effects of the treatments is discussed In our study the patients were divided into three groups by the degree of hearing loss (Table II) In the total hearing loss type patients the recovery rate and the average pure tone improvement were significantly higher in SGB and OHP treated group than in the medically treated group

Age is also an important factor Patients under 40 years old almost always improved even when the initiation of treatment was delayed Of 9 improved cases (Table I group 7), seven treated later than four weeks after onset were under 40 years old

Haug et al (1976) reported the effects of SGB and Yanagita (1974) and Yanagita et al (1976) reported good results following treatment with OHP However the incidence of significant pure tone improvement in patients treated two weeks after onset was very low Our study shows that there is a 100% chance that patients will recover over 10 dB and a

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LONG-TERM RESULTS OF OPEN CAVITY AND TYMPANOMASTOID SURGERY OF THE CHRONIC EAR

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Abstract The results of surgery for chronic middle ear infection in 307 patients (347 ears) are reported. All ears were operated on according to one of two procedures ( ) the operation cavity was left open after removal of the posterior canal wall, or ( ) tympanomastoidectomy with intact canal wall was done. When necessary removal of disease in the tympanum with or without reconstruction of the sound-conducting mechanism of the middle ear was performed. Cholesteatoma was present in 56% of the ears. Open cavity surgery with no reconstruction was done in 53% In the remaining 47% tympanomastoidectomy was performed and in approx. half of these ears reconstruction was undertaken. The postoperative follow-up period averaged 9 years. Residual cholesteatoma was found in 9% In 4% of the ears undergoing tympanomastoidectomy retraction pocket cholesteatoma developed. During the follow-up period 13% of the ears required revision surgery. Nineteen per cent of the ears with open cavities were discharging and in addition, crusts were observed in 13% In the tympanomastoidectomy group discharging ears were seen in 76% As a rule hearing was not affected by surgery. The mean elevation of the pure tone threshold was 6.5 dB on account of sensorineural loss, while the speech reception threshold shift was 11 dB. The results testify to the importance of removing the posterior canal wall in ears with chronic middle ear infection associated with cholesteatoma.

Surgical treatment has been used for chronic middle ear infection for a little more than 100 years. To begin with surgeons were content to open the antrum only and maintain drainage of the mastoid process in various ways but it was not very long before the radical operation with open cavity was introduced. With various modifications this method has been the favoured procedure for some 80 years. The open cavity needs regular postoperative care however and aided by the development of microsurgery otologic surgeons have made attempts at improvements.

Efforts at eliminating the large open cavity resulted in the introduction of two methods referred to as the canal wall down and intact canal wall methods. Their foremost advocates have been T Palva (1962) and Jansen (1963). In Palva's opinion the bony wall of the ear canal must be removed in radical surgery of a chronically infected ear with cholesteatoma. Jansen again claims that the bony wall can be retained in such an ear with the combined approach tympanoplasty (CAT). Palva obliterates the cavity with a musculoperiosteal flap and reconstructs the ear canal with cortical bone chips bone paté and fascia while Jansen considers an air filled cavity necessary.

For the last 10 years the intact canal wall method has been the favored procedure all over the world. At the first international conference on cholesteatoma held in Iowa City in 1977 most of the participants, however reported a high frequency of cholesteatoma recurrence following this operation and its popularity is steadily declining.

Comprehensive studies on long term results of surgery for chronic middle ear infection are rare. Therefore this study on open radical cavities and tympanomastoidectomies has been carried out, the aim being to provide data with which surgical results obtained with newer methods can be compared in the future when long-term results are available.

MATERIAL AND METHODS

The material consists of patients who underwent surgery for chronic middle ear infection

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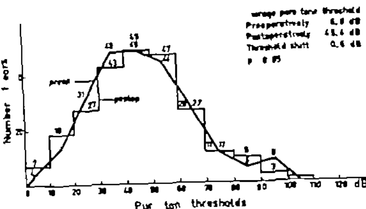


Fig. 1. Pre-and postoperative pure tone thresholds. Short-term results obtained in 41 ears.

operations such as obliteration of the cavity enlargement of the external meatus closure of a fistula behind the ear etc

Complications

Complications following surgery were rare In one ear an opening developed in the posterior semicircular canal in one ear the stapes were inadvertently removed and in two ears dura and arachnoid ruptures occurred In one case so much bone was removed that a brain prolapse occurred into the cavity In another ear a gauze strip was mistakenly left in the posterior part of the open cavity and it was only after its removal 6 years later that the ear ceased to discharge

In 5 cases massive infection of the operative wound developed resulting in 3 ears in retroauricular fistulae Perichondritis occurred in the concha of one ear In 3 cases the facial nerve became paralytic though in of them it regained its function

Cholesteatoma recurrence

At primary surgery cholesteatoma was found in 56% of the ears In 9% of the ears in which primary surgery had revealed cholesteatoma, cholesteatoma recurred necessitating a second operation In addition, cholesteatoma was found at secondary surgery in 4% of ears which at primary surgery had been free from cholesteatoma.

Labyrinth fistula

Fistula of the labyrinth was seen in 8% of all ears included in the study Ninety two per cent of the fistulae occurred in cholesteatoma ears and the remaining 8% developed in ears which had earlier been subjected to surgery In all instances the fistula was seen in the lateral semicircular canal Twelve per cent of the ears were deaf prior to surgery and 4% became so as a result of the operation In the rest of the ears with fistulae hearing was generally speaking unchanged after surgery The cholesteatoma lining covering the fistula was removed in all but 3 cases In 2 of these cases, the operation cavity was smooth and uninfected when seen at the follow-up examination while in the third infection and also cholesteatoma were present in the cavity

Hearing result

Five (2%) of the 282 ears examined at the follow-up examination were preoperatively deaf i e did not hear at 120 dB HL at 0.5 1 and kHz by air conduction Three ears (1%) heard all these frequencies prior to surgery but did not do so postoperatively Preoperatively their pure tone threshold by air conduction was on average 81 dB In addition, 7 ears (3%) lost the ability to hear at 120 dB HL at one or two frequencies in the speech area.

When examined at the follow-up 12% of the ears were found not to hear at 120 dB HL at 4 kHz The percentage of ears preoperative

in the Department of Otolaryngology Helsinki University Finland in the years 1964–1968 The study includes the patients who had been operated on according to either of the following methods (i) the posterior bony wall of the ear canal was removed and the operative cavity left open or (ii) a tympanomastoidectomy was performed in which the canal wall was left intact When necessary disease in the tympanum was removed and in part of the ears the sound-conducting mechanism of the middle ear reconstructed

The entire material comprises 307 patients In 40 patients both ears were operated on which gives a total of 347 ears Of these 347 282 were examined at a follow up examination while data on 17 patients was obtained by letter Nineteen patients had died and 29 patients did not respond to the request to attend the follow up examination The mean observation period was 9 4 years

At the follow-up examination the radical operative cavity and the middle ear were examined when necessary with the aid of a microscope If moisture or pus was present a swab for bacterial culture was taken In the audiometric test pure tone thresholds (ISO) by air and bone conduction were recorded with appropriate masking whereupon the speech reception threshold and maximal discrimination score were determined The average pure tone threshold was calculated as the mean of the thresholds for the frequencies of 0 5 1 and 2 kHz In addition the threshold values at 4 kHz by air and bone conduction were evaluated separately

The statistical relevance of the results of the audiometric testing was tested by the bilateral t test (Student s t test)

RESULTS

All ears included in the study were discharging prior to the operation either continuously or intermittently If the patient had to wait for surgery the ear was as a rule treated with antibiotics and/or ear drops and mechanically cleansed

Open cavity surgery

In the entire material open cavity radical mastoid surgery was performed on 183 patients (53 %) In these ears cholesteatoma was present in 78 % 90 % of the ears were discharging prior to surgery 84 % at the time of surgery and 19 % at the time of the follow-up examination In addition slight moisture under the secretion crusts was observed in 13 % of the ears subjectively the ears were dry but needed regular cleansing

In 5 % of the ears revision surgery had to be performed on account of residual cholesteatoma which constitutes 6 % of the ears with cholesteatoma at the primary operation.

Tympanomastoidectomy

Tympanomastoidectomy was performed in 164 ears (47 %) of the entire material In these ears the bony wall of the external meatus was left intact The sound-conducting mechanism in the middle ear was reconstructed in 57 % of these ears In approx half of the ears the ossicular chain was intact however and the tympanic surgery consisted only in a myringoplasty Cholesteatoma was present in 30 % of the ears and 73 % had been discharging preoperatively 60 % of the ears were discharging at the time of surgery and 26 % at the time of the follow up examination Of the cholesteatoma ears 15 % were reoperated on because of cholesteatoma. In addition a retraction pocket cholesteatoma developed in 8 ears (4 %) without cholesteatoma at primary surgery

Revision surgery

During the follow up period 17 % of the ears in the entire material were reoperated on Surgical procedures were carried out twice in 13 % thrice in 3 % and four times in 1 % of the ears In the majority of these cases 13 secondary surgery was done because of persistent infection in the ar In some of these ears measures to improve hearing were performed at the same time The rest of the revision surgery was planned as second-stage

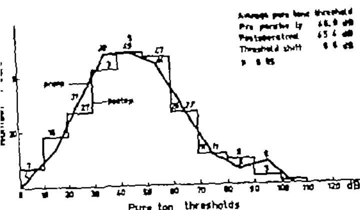


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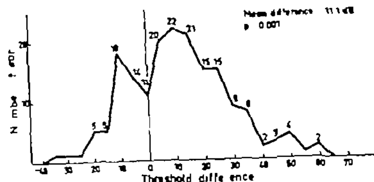


Fig. 3. The difference between pre and postoperative speech reception thresholds. Long-term result obtained in 178 ears

In comparison, the percentages of postoperatively infected ears are fairly high in the present material. The explanation may be sought in the operative technique: on the one hand, the bone work was not sufficiently radical, and, on the other, removal of infected tissue in the tympanum was not sufficient. As the operations were performed at a teaching clinic, the skill and experience of the surgeons varied considerably. The cautious operative technique had the advantage, however, that surgical accidents were rare.

Residual cholesteatoma was seen in 9% of the operated ears. In the group operated on according to the 'canal wall down' technique, the cholesteatoma recurrence rate was only 6% as against 15% in the group in which 'canal wall intact' was employed. In addition cholesteatoma was found in 4% of the ears which at primary surgery had been free of cholesteatoma. The total cholesteatoma frequency during the follow-up period was thus ...%.

In the literature the reported postoperative cholesteatoma frequency shows great variations. Sadé (1977) recorded cholesteatoma recurrence following 'intact wall' operation in ...% of the patients and the recurrence rate was as high as 48% if at primary surgery the cholesteatoma was large or the patient under 20 years of age. Cody & Taylor noted recurrent and/or residual cholesteatoma in 35%, in addition to which precholesteatoma in the form of an attic retraction pocket or a facial recess retraction pocket or both occurred in 20%. Smyth (1977) reported postoperative cholesteatoma in 35%, Glasscock (1977) in 26%, while Jansen (1977) reports such cholesteatoma in 3% of his patients.

With canal wall down surgery results are consistently better. Cody & Taylor (1977) found postoperative cholesteatoma in 16%, A. Palva et al. (1977) in 3% of children under 16 years of age, Lee & Schuknecht (1971) in 2.2% and T. Palva et al. (1977) in 2% of their patients.

In light of these figures and considering the experience of the surgeons, the open cavity results in the present series must be considered satisfactory. They are distinctly better than most of the well-controlled figures in 'intact canal wall' technique series reported by highly experienced surgeons. The long observation time probably increases the cholesteatoma frequency in this material.

Fistula of the labyrinth occurred in 8% of all ears and in 13% if only ears with cholesteatoma are taken into account. Other authors have reported similar figures: Sheehy et al. (1977) 10%, Wayoff & Friot (1977) 11% and T. Palva et al. (1977) 8%.

In 2 of the 3 ears of the present material in which cholesteatoma lining was left covering the fistula, this area was smooth and uninfected when seen at the follow-up examination, while in the third case it was infected and covered with cholesteatoma.

Sheehy (1970), T. Palva et al. (1971) and

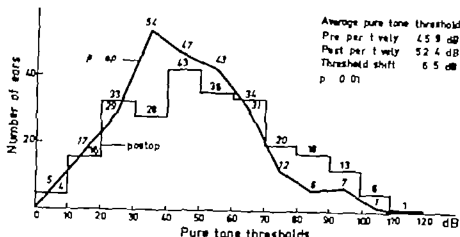


Fig 2 Pre and postoperative tone thresholds Long-term results obtained in 25 ears.

ly deaf to this frequency was 5 while 6% of the ears which had heard this tone preoperatively had lost this range by the time of the follow up examination

Figs 1 and 2 show the mean pure tone thresholds for air conduction The preoperative thresholds are compared with the values obtained after both short and long observation periods It appears that generally speaking surgery did not cause impairment of hearing the preoperative curve runs almost parallel with the short term results However the long term results show a statistically significant deterioration of hearing the mean preoperative level was 45 9 dB as against 52 4 dB postoperatively Thus the mean threshold shift was 6 5 dB

Fig 3 illustrates the differences between pre and postoperative speech reception thresholds The long term results show a highly significant impairment the mean threshold shift being 11 1 dB The short term results did not show any change in speech reception threshold

A highly significant sensorineural hearing loss at 4 kHz mean 12.2 dB was observed The corresponding short term threshold shift was only 4 dB In order to determine the effect of the patient s age on postoperative hearing the threshold shifts of the 50 youngest and the 50 oldest patients were compared The mean ages of these two groups were 21 and 55 years In the long term results the corresponding pre and postoperative differences were – dB and –15 dB indicating that hearing in the older group deteriorated on average twice as much as in the group of younger patients during the 9-year follow-up period

DISCUSSION

During the follow up period revision surgery was performed in 13% of all ears examined In spite of this removal of disease was found to be unsuccessful in 19% in the open cavity group Crusts had collected in the cavity in a further 13% but subjectively these ears were dry In the tympanomastoidectomy group revision surgery was done in 26% of the ears

Results of surgery vary considerably in the literature depending on the severity of the middle ear infection cholesteatoma frequency surgical techniques duration of operative procedures and the skill of the surgeon Furthermore the criteria for presence of middle ear infection have yet to be agreed upon Thus Palva et al (1968) reported postoperative infections in 20% of the patients in their open cavity series 13% of the infections being of short duration however Lee & Shuknecht (1971) reported 16% and in addition crusts were observed in 14% of the patients Pfaltz et al (1975) 7% A Palva et al (1977) 8% in children with cholesteatoma T Palva et al (1977) 10% and Glasscock (1977) finally a postoperative infection rate of 14%

Tympanomastoidektomie unterzogen wurden, entwickelte sich ein Cholesteatom-Retraktionsbeutel. Während der Beobachtungsperiode erforderten 13% der Ohren einen erneuten chirurgischen Eingriff. Bei neunzehn von hundert Ohren mit offenen Kavitäten kam Ausfluß vor und außerdem wurden bei 13% der Fälle Krusten festgestellt. In der Tympanomastoidektomie-Gruppe kam bei [illegible]% der Fälle Vereiterung der Ohren vor. Im allgemeinen wurde das präoperative Gehör nicht durch chirurgische Eingriffe beeinträchtigt. Der Mittelwert der reinen Tonschwelle belief sich aufgrund des sensorineuralen Verlustes auf 6,5 dB, während sich die Sprachhörschwelle auf 1 dB verschlechterte. Die Ergebnisse sprachen für eine Entfernung des hinteren Ohrkanals für Ohren mit chronischer Mittelohrentzündung, wobei gleichzeitig Cholesteatome auftreten, deutlich.

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Gacek (1973) have recommended removal of cholesteatoma lining overlying the fistula This removal should be the last step before proceeding to reconstruction of the sound conducting mechanism in the middle ear and be performed by an experienced surgeon so that the inner ear is not damaged

An adequate removal of disease must be considered the guiding rule in operative treatment of chronic middle ear infection especially in the presence of cholesteatoma A prerequisite for successful removal is a good view and as a rule the bony posterior and superior canal wall has to be removed It is always necessary for inexperienced surgeons to remove the bony wall totally if cholesteatoma is present The cavity can be filled with flap *ad modum* Palva With growing experience reconstruction of the ear canal and total obliteration of the operative cavity can be undertaken as a routine measure An operation at which the ear canal is left intact (CAT) cannot be recommended in ears with cholesteatoma as the long term frequency rate of residual cholesteatoma is too high even when the operation is performed by highly experienced surgeons When the bony wall of the ear canal is left intact in ears without cholesteatoma a sufficiently long observation time will reveal that cholesteatoma develops in a number of ears as an adhesive process draws the squamous epithelium of the ar canal and drum into the mastoid cavity This can be avoided if the operative cavity is primarily obliterated

Myringoplasties were performed as early as in the 19th century but it was not until the last 25 years that tympanoplastic procedures could be performed At first there was a great deal of optimism in regard to the improvement
1 in hearing achieved by these operations Gradually lasting hearing improvement has become rare and conversely studies reporting poor hearing results much more common Jansen claimed that 77% of ears operated on according to the CAT technique had average hearing levels of 30 dB or better while only 6% had a hearing level poorer than 40 dB On the other hand Cody & Taylor (1977) found that hearing results were poor with all of the three operative methods they used and only slightly better with the CAT method. A Palva et al (1977) reported deterioration of hearing from the 34 to the 37 dB level on average in their series of children with cholesteatoma

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Tympanomastoidektomie unterzogen wurden, entwickelte sich ein Cholesteatom-Retraktionsbeutel. Während der Beobachtungsperiode erforderten 13% der Ohren einen erneuten chirurgischen Eingriff. Bei neunzehn von hundert Ohren mit offenen Kavitäten kam Ausfluß vor und infiziert wurden bei 13% der Fälle Krusten festgestellt. In der Tympanomastoidektomie-Gruppe kam bei 3% der Fälle Verengerung der Ohren vor. Im allgemeinen wurde das präoperative Gehör nicht durch chirurgische Eingriffe beeinträchtigt. Der Mittelwert der reinen Tonschwelle belief sich aufgrund des sensorineuralen Verlustes auf 6,5 dB, während sich die Sprachhörschwelle um 11 dB verschlechterte. Die Ergebnisse sprachen für eine Erneuerung des hinteren Ohrkanals für Ohren mit chronischer Mittelohrentzündung, wobei gleichzeitig Cholesteatome auftreten, deutlich.

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Gacek (1973) have recommended removal of cholesteatoma lining overlying the fistula This removal should be the last step before proceeding to reconstruction of the sound conducting mechanism in the middle ear and be performed by an experienced surgeon so that the inner ear is not damaged

An adequate removal of disease must be considered the guiding rule in operative treatment of chronic middle ear infection especially in the presence of cholesteatoma A prerequisite for successful removal is a good view and as a rule the bony posterior and superior canal wall has to be removed It is always necessary for inexperienced surgeons to remove the bony wall totally if cholesteatoma is present The cavity can be filled with flap *ad modum* Palva With growing experience reconstruction of the ear canal and total obliteration of the operative cavity can be undertaken as a routine measure An operation at which the ear canal is left intact (CAT) cannot be recommended in ears with cholesteatoma as the long-term frequency rate of residual cholesteatoma is too high even when the operation is performed by highly experienced surgeons When the bony wall of the ear canal is left intact in ears without cholesteatoma, a sufficiently long observation time will reveal that cholesteatoma develops in a number of ears as an adhesive process draws the squamous epithelium of the ar canal and drum into the mastoid cavity This can be avoided if the operative cavity is primarily obliterated

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CRITICAL BANDS FOLLOWING THE SELECTIVE DESTRUCTION OF COCHLEAR INNER AND OUTER HAIR CELLS

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Abstract Critical bandwidths and absolute intensity thresholds were measured in cats before and after kanamycin treatment which induced selective inner and outer hair cell losses Hair cell losses were measured from cochleograms constructed from surface preparations of the organ of Corti Results suggested that for the test frequencies and stimulus intensities employed critical bandwidths were not affected for frequencies tonotopically located in cochlear regions where only outer hair cells were lost Critical bands were widened or not measurable only when inner hair cell losses exceeding 40% were also associated with complete loss of outer hair cells The experiment suggests that cochlear frequency selectivity can be mediated by inner hair cells alone

It has been suggested that inner and outer hair cells of the cochlea may play differential roles in frequency coding Evidence has normally come from either physiological or behavioural experiments in which outer hair cells have been selectively destroyed using an ototoxic drug and the consequent cochlear function observed relates to inner hair cell activity alone

The physiological study of Kiang et al (1970) showed broadened auditory nerve frequency tuning curves in cats following selective destruction of outer hair cells by means of kanamycin sulphate Since then Evans (1975*a*) has argued for an inner–outer hair cell interaction to explain frequency sharpening at a cochlear level and has reported high threshold broad tuning curves in cochleae subjected to various kinds of trauma (Evans 1972 Evans 1974 Evans & Klinke 1974) Similarly Zwislocki (1975) has proposed a model of hair cell interaction to explain cochlear frequency sharpening

Behavioural experiments are less numerous. Selective outer hair cell destruction consistently results in elevations of behavioural auditory thresholds of up to 50 dB (Schuknecht, 1953 Ryan & Dallos 1975) Few studies however have examined the effects of outer hair cell destruction on behavioural measures of frequency coding at the cochlear level Dallos & Ryan (1975) and Dallos et al (1977) found that frequency selectivity as reflected in psychophysical tuning curves was unaffected by selective outer hair cell destruction in the chinchilla It is however possible that psychophysical tuning curves reflect processing in higher auditory centres in addition to frequency selectivity at a cochlear level (Dallos et al 1977)

Nienhuys & Clark (1978) found that frequency discrimination in cats remained unaltered for frequencies related to regions where all outer hair cells were destroyed but 50% of inner hair cells were intact However frequency discrimination is the ability to discriminate two non simultaneous pure tones differing only in frequency (Cardozo 1974) and is a different task from frequency selectivity which is the ability to separate out components of a simultaneous complex signal (Evans 1975*b*)

Therefore to further examine the effect of outer hair cell elimination on cochlear frequency selectivity critical bandwidths were

behaviourally measured in cats before and after selective outer hair cell destruction using kanamycin sulphate The critical bandwidth has been commonly viewed as a measure of cochlear frequency selectivity (Evans 1975*a* Sharf 1970 Zwicker 1970) and in contrast to psychophysical tuning curves extensive information is available concerning critical bands in both humans and animals

METHOD

Plan of the experiment

Monauralized cats were trained to criterion performance in the conditioned suppression of an ongoing licking response to the presentation of a series of tone bursts Each cat s behavioural absolute intensity threshold at frequencies 1 4 8, 10 12 and 16 kHz were measured twice over 12 days Critical bandwidths were behaviourally estimated at central frequencies of 1 4 8 10 and 12 kHz over 30 days. Cats were then treated with kanamycin sulphate (Kantrax) 200 mg/kg/day (i.m) for 10 days, followed by a 14-day recovery period. Post-drug absolute thresholds were then measured (12 days) followed by post-drug critical bandwidth measurements (36 days) Finally all absolute thresholds were measured again (12 days) Immediately before sacrificing each cat, cochlear microphonic thresholds were measured. Surface preparations of the organ of Corti were then prepared and cochleograms constructed from them

Subjects

Four adult cats were selected for this experiment (Nos 1 2 3 4) The contralateral middle and inner ear of each cat were surgically destroyed. After removing the ossicular chain the middle ear was filled with dental cement. The inner ear was then perfused with absolute alcohol which was applied through the round window and removed by suction from a hole drilled in the apical turn.

Conditioning apparatus

A conditioning box was placed inside a sound attenuated chamber with sound-absorbent inner walls The box was 58 cm high 41 cm long and 20 cm wide It had no ceiling and its floor consisted of 5 mm diameter brass bars set 5 mm apart through which brief shocks of 4 mA at 120 volts could be applied to the cats feet. A licking spout was fixed at one end of the box 10 cm above floor level and milk was pumped by an infusion apparatus at a variable rate around 7 ml/min The speaker was mounted so that its axis lay 7 cm to the left of the cat s test ear The opposing wall of the box was constructed of half-inch chicken wire All remaining inside walls of the box were lined with thick woollen carpet.

Absolute intensity threshold testing

Absolute intensity thresholds were determined using the conditioned suppression procedure Cats were required to lick steadily in the absence of a test stimulus and to suppress licking in the presence of a test stimulus The essential first step was to establish a baseline ongoing licking response Cats were fluid deprived and maintained on dry pellet cat food A continuous milk flow of about 7 ml/min soon achieved a regular lick rate of 22 to 38 licks per 10 sec in all cats Training was continued until all cats licked steadily for 20 min over 4 consecutive days

The test tone was presented for periods of 10 sec during which ten sinusoidal pure-tone bursts of 700 msec duration with a rise–fall time of 2.5 msec and separated by 300 msec silent intervals were given. At the end of each 10-sec period, a brief unavoidable electric shock was applied to the cat s feet. This sequence constituted a test trial. A circuit was designed which counted all cats licks

To produce tone bursts the output of a Datapulse 410 generator was gated by a Grason-Stadler 829E electronic switch coupled to a Grason-Stadler 4711 interval timer which was triggered externally by computer For test

frequencies of 1 and 4 kHz a Philips AD5060 speaker was used and for 8 10 12 and 16 kHz a Richard Allen DT20 treble speaker was employed Sound pressure levels were measured with a Brüel & Kjær 4134 amplifier and half-inch condenser microphone placed beside the ear of a cat cadaver simulating a licking cat

During the 10-sec period of tone bursts cessation of licking in anticipation of the electric shock indicated that the cat had heard the tones During suppression training tone bursts were presented at 60 dB SPL

The suppression response was measured by calculating the suppression ratio $((P-D)/P)$ where P equals the number of responses during the 10-second period immediately preceding the warning stimulus and D equals the number of responses occuring during the warning stimulus The suppression response was considered to have been established when the mean suppression ratio for test trials was 0 75 or higher on each of three successive daily sessions Test trials were separated by intervals of 60 to 90 seconds and interspersed with sham trials as recommended by Smith (1970)

Thresholds were measured using a response adjustment technique with discrete stimulus stepping under experimenter control If a response occurred stimulus intensity was reduced by 10 dB if not intensity was increased by 10 dB Cats detection performance soon came to equilibrium near threshold and step sizes were reduced to 5 dB When six consecutive response reversals occurred at 5 dB step sizes the threshold was taken as the median of the two intensities presented at equilibrium Thus a single threshold was measured in a daily session involving about 20 test trials The absolute threshold for each frequency was then taken as the mean of two thresholds measured in two daily sessions

Critical bandwidth measurements

Critical bandwidths were behaviourally estimated at test frequencies of 1 4 8 10 and 12 kHz using the narrow band masking method. This method finds the masked threshold of pure tone signals which are centred in a series of narrow bands of noise of variable width and constant total power As the noise bandwidth is increased power is added to the noise band, increasing its masking effect until the bandwidth exceeds the critical band (Greenwood 1961*a*) That bandwidth beyond which masked thresholds decline at 3 dB per octave bandwidth may be taken as the critical one. This technique has previously been employed and found adequate by Pickles (1975)

At each test frequency six bandwidths were used in random order Bandwidths increased in octave steps so that they ranged well above and below the critical bandwidths found for cats by Pickles (1975) A Hewlett Packard 8057A generator produced white noise which was passed through a variable low pass filter (Krohn Hite 3750R) with a high-frequency cutoff of 24 dB per octave The result was modulated by a carrier sinusoid in a balanced modulator (Datapulse 410 function generator) producing bandpass noise The modulated signal was passed through a Brüel & Kjær 2120 frequency analyser acting as a high-pass filter and also controlling the total noise intensity of the final noise band The accuracy of the output noise band at each frequency was checked using equipment described earlier All noise bands around 1 and 4 kHz were produced at 50 dB SPL with an accuracy of ±4 dB and around 8 10 and 12 kHz with an accuracy of ±5 dB

The technique for behavioural measurement of masked thresholds was the same as that used for absolute thresholds except that noise bands were now added As described in the Results Section all cats showed a flattened masked-threshold bandwidth function at 12 kHz following drug treatment This may have occurred because the drug induced threshold elevations had raised all masked thresholds including those within the critical band to a maximal level To check this possibility all masked thresholds at 12 kHz were retested at

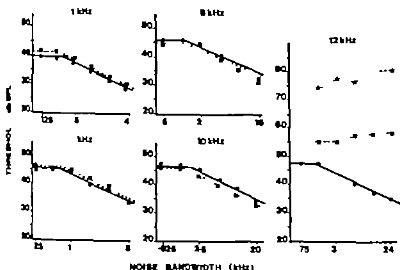


Fig. 1. Masked threshold-noise bandwidth plots and best-fitting two-part regression lines for cat 3 in the pre-drug (●—●) and post-drug (■ ■) conditions. Retested masked thresholds for 12 kHz with noise increment of 30 dB are also shown (closed stars, dotted lines).

noise intensity levels which were raised from 50 dB SPL by an amount equivalent to each cat's threshold elevation at 1 kHz. A Quad 202 amplifier was inserted to enable pure-tone burst levels to be raised by 20 dB SPL for this procedure.

Surface preparations

Immediately following final behavioural testing, cats were anaesthetized with pentobarbital sodium (Sagatal 60 mg/kg i.p.) and a silver–silver chloride electrode was placed on the round window membrane of the left ear. Cochlear microphonic thresholds were measured for 30 msec pure tone bursts at 1, 3, 4, 8, 10, 12, 16 and 20 kHz for cats 1, 3 and 4. Finally, all cats were perfused so that surface preparations could be made according to the technique described by Engström et al. (1966). Cochleograms were drawn by counting all hair cells in 0.0782 mm segments under calibrated differential interference microscopy.

RESULTS

Absolute intensity threshold measurements

The mean pre-drug absolute thresholds at each frequency across all cats compared favourably with those found by Elliott et al. (1960) in normal cats, ranging from −8 dB SPL at 1 kHz to −11.5 dB SPL at 10 kHz. The mean difference between the two post-drug threshold values across all cats was only 0.75 dB SPL (S.D. 3.0 dB SPL). This result suggested that the kanamycin-induced threshold shifts had stabilized during the 14-day waiting period following drug treatment, and no significant subsequent elevations occurred during the 36 days of post-drug initial bandwidth testing. Table I shows the absolute intensity threshold shifts at each test frequency which resulted from kanamycin treatment. These shifts were calculated as the difference between the pre-drug threshold and the mean of the two post-drug thresholds for each cat at each test frequency.

Critical bandwidth measurements

Masked thresholds at each masker bandwidth for each test frequency were plotted for each cat. Fig. 1 illustrates these for cat 3. Critical bandwidths were calculated using Pickles' (1975) technique. That is, for masker bandwidths smaller than the critical bandwidth, masked thresholds should be unaltered, producing an expected straight-line regression function of zero slope. For masker bandwidths wider than the critical bandwidth, masked thresholds should reduce at 3 dB per octave masker bandwidth. Therefore, for all cats at

Table I *Post-drug absolute intensity threshold shifts and outer and inner hair cell losses pre-drug and post-drug critical bandwidths for all cats at each test frequency (1 4 8 10 and 12 kHz)*

	Test Frequency (kHz)				
	1	4	8	10	1
Cat 1					
Intensity threshold shift (dB)	1	1	25.5	33.5	39
Inner hair cell loss (%)	0	0	20	70	70
Outer hair cell loss (%)	0	50	100	100	100
Pre-drug critical bandwidth (kHz)	0.380	0 700	1 100	1 400	1 700
Post-drug critical bandwidth (kHz)	0.280	0 600	1 100	1 [illegible]50	
Cat 2					
Intensity threshold shift (dB)	−2	12	18.5	36.5	39
Inner hair cell loss (%)	0	0	10	20	90
Outer hair cell loss (%)	0	40	60	100	100
Pre-drug critical bandwidth (kHz)	0.410	0.5[illegible]0	1 450	1 450	1 700
Post-drug critical bandwidth (kHz)	0 410	0.560	1 100	1.250	
Cat 3					
Intensity threshold shift (dB)	3	16	[illegible]6	26.5	30 5
Inner hair cell loss (%)	0	0	0	10	60
Outer hair cell loss (%)	0	50	100	100	100
Pre-drug critical bandwidth (kHz)	0.320	0 620	1.200	1.500	1.350
Post-drug critical bandwidth (kHz)	0.[illegible]60	0.710	1 100	1.300	
Cat 4					
Intensity threshold shift (dB)	4 5	15	24	30	34
Inner hair cell loss (%)	0	0	0	10	40
Outer hair cell loss (%)	0	60	100	100	100
Pre-drug critical bandwidth (kHz)	0.370	0.620	1 400	1.200	1 [illegible]00
Post-drug critical bandwidth (kHz)	0 420	0 700	1 600	1 125	-

all test frequencies in the pre-drug condition the expected two-part function was tested for statistical acceptability Within the critical band the mean slope of −0 24 dB per octave (S D 0 63 dB per octave) was not significantly different from zero slope (t=1 699 19 d f) Beyond the critical band the mean slope of −3 24 dB per octave (S D 0 62 dB per octave) was not significantly different from −3 dB per octave (t=1 738 19 d f) The resultant pre drug critical bandwidth measurements are shown in Table I

In the post-drug condition masked threshold masker bandwidth functions showed the expected two-part function at each test frequency except 12 kHz in all cats At 1 4 8 and 10 kHz the mean slope of regression lines within the critical band was 0 13 dB per octave (S D 0 72 dB per octave) not significantly different from zero slope (t=0 696 15 d f) Beyond the critical band the mean slope was −3 21 dB per octave (S D 0.48 dB per octave) not significantly different from −3 dB per octave (t=1 717 15 d f) Resultant postdrug critical bandwidths are also shown in Table I

The critical bandwidth could not be found for any cat at 12 kHz in the post-drug condition as masker bandwidth-threshold functions did not show the expected two-part function This suggested that at 12 kHz in the post drug condition the critical bandwidth function was either widened beyond the widest bandwidths tested (24 kHz) or completely obliterated This effect persisted when all masker intensity levels at 12 kHz test frequency were

creased for each cat from 50 dB by an amount equivalent to that cat's absolute intensity threshold elevations Fig. 1 demonstrates this for cat 3

F ratios were calculated for critical bandwidth measures across all cats at test frequencies of 1 4 8 and 10 kHz to test for significant differences between the pre-drug and the post-drug conditions No *F* ratio reached significance at any test frequency ($\alpha = 0.05$ 1 6 d.f.).

It is possible that while critical bandwidths did not alter following drug treatment masked threshold levels were altered as a result of signal-to-noise ratio variations This possibility was excluded by calculating the mean difference between pre and post-drug masked thresholds for each cat at each frequency other than 12 kHz. This mean ranged from as low as 0 08 dB (S D 0 76 dB) for cat 4 at 1 kHz to a maximum of 4 12 dB (S D 2 14 dB) for cat 1 at 8 kHz.

Histological correlates

Table I shows the percent inner and outer hair cell losses for each cat at each test frequency The losses ranged from zero in all cats at cochlear regions corresponding to 1 kHz to total hair cell loss in all cats for regions corresponding to 16 kHz.

Table I reveals a good correspondence between behavioural and histological measures of hair cell lesions Behavioural threshold elevations increased with the degree of outer hair cell loss in all cats. For cats 3 and 4 at 8 kHz where complete selective outer hair cell destruction occurred threshold elevations were 26 dB and 24 dB respectively Threshold elevations increased further when inner hair cells also became involved in the lesions This result is consistent with findings by Schuknecht (1953) in cats and Ryan & Dallos (1975) in chinchillas

Furthermore critical bandwidths were not affected for any test frequencies associated with cochlear regions which suffered up to total outer hair cell loss Critical bandwidths were affected however when inner hair cell losses of 40% or more occurred in addition to outer hair cell loss When this occurred masked threshold bandwidth functions were flattened suggesting destruction or widening of the critical band mechanism

DISCUSSION

The results of this experiment suggest that outer hair cell elimination in a cochlear region does not affect critical bandwidths for those test frequencies tonotopically located in the affected cochlear region Rather cochlear frequency selectivity is affected only when more than 40% of inner hair cells are also involved in the lesion This conclusion depends upon certain assumptions

First it was assumed that (*a*) hair cells appearing normal under differential interference microscopy were functionally normal (*b*) hair cell lesions were stable and did not deteriorate during the 36 days of post-drug initial bandwidth testing, and (*c*) ototoxic effects were localized at the hair cell level leaving associated neural elements unaffected Assumptions (*a*) and (*c*) were similar to those made by Ryan & Dallos (1975)

A detailed knowledge of the effect of aminoglycoside antibiotics on hair cell activity and intracellular histopathology is not yet available However Wersäll (1973) reported that intracellular changes occur most rapidly immediately after drug treatment in guinea pigs followed by a period of relative stability Accordingly Ryan & Dallos (1975) and the present study have found stable behavioural auditory thresholds around 7–14 days following drug administration In addition the present study found stable thresholds again at 36 days after initial post-drug testing. These findings lend support to assumption (*b*) above When survival times extend beyond about 3 months long-term hair cell degeneration may be expected (Aran & Darrouzet 1975) accompanied by secondary degeneration of the spiral ganglion and the acoustic nerve (Ostyn & Tyberg-

hein 1968) which does not occur at earlier stages (Galasińska-Pomykol et al 1975) Therefore the present study was designed to be completed before such long term changes took place supporting assumption (*c*) Assumption (*a*) is supported in part by the correlation between auditory threshold elevations and the degree of hair cell loss seen in Table I This assumption may not be critical in any case since the results showed that the inner hair cells were functioning at least well enough to mediate critical bandwidths normally

Second it was assumed that critical bandwidths reflect cochlear frequency selectivity and are not primarily elaborated by higher auditory centres Since Fletcher (1940) first described the critical band its locus has been assumed to be at the cochlea (Sharf 1970) This assumption has been supported by findings that the critical bandwidth appears as an exponential function of distance along the basilar membrane and that critical bandwidths correspond to regular basilar membrane distance (Greenwood 1974 Pickles 1975) Zwislocki (1965) has postulated that critical bandwidths correspond to equal numbers of cochlear neurons Similarly critical bandwidths have been reported to be proportional to frequency difference limens (Fletcher 1940 Sharf 1970 Zwicker 1970) although evidence for this is conflicting (Moore 1974 Pickles 1975 Watson 1963)

The most significant evidence for the view that the critical band mechanism resides in the cochlea comes from studies which make direct comparisons between critical bandwidths and recorded effective bandwidths of single auditory nerve fibres Evans (1975*a*) and Evans & Wilson (1971) showed that the effective bandwidth of frequency tuning curves in cats agree well with critical bandwidths measured in man by Zwicker et al (1957) However when Pickles (1975) compared critical bandwidths in cats with the neural data of Evans & Wilson (1971) of the same species he found that the critical bandwidths were consistently wider than the nerve fibre bandwidths. This result persisted when Pickles & Comis (1976) measured nerve fibre bandwidths using acoustic stimulation similar to the narrow-band masking technique employed in the psychoacoustic study

However mean pre-drug critical bandwidths across cats in the present study were consistently smaller than Pickles (1975) reported at all frequencies higher than 1 kHz. Therefore the present mean critical bandwidth values show overlapping with the neural bandwidth data of Evans & Wilson (1971) which did not occur for Pickles mean critical bandwidth data It may be that differing behavioural techniques account for the discrepancy between these and Pickles data. Seaton & Trahiotis (1973) suggested that frequency resolving power measurements in animals can depend upon the behavioural technique employed Certainly cats critical bandwidths still need to be measured with techniques other than narrow-band masking Nevertheless it appears that the difference between physiological and psychophysical bandwidths may not be as great as Pickles (1975) reported

Further evidence for a cochlear locus of the critical band should derive from observations of impaired cochleae In human studies evidence of disturbed critical bands in impaired cochleae has been confusing (de Boer 1959 de Boer & Bouwmeester 1974 Martin 1974) However by using animals the present study could directly compare behavioural and histological data Results showed that the critical band mechanism was disturbed only in cochlear regions suffering significant hair cell lesions This suggests a tonotopic location of critical bands as postulated by Greenwood (1961*b*) and would not be expected if critical bands were primarily elaborated in auditory centres higher than the cochlea

A potential difficulty in the procedure of this experiment is the accuracy of the frequency to-place relationships inferred for each cochleogram on the basis of organ of Corti length according to the formula provided by Kiang

et al (1970) It may be that these relationships do not hold for all organ of Corti lengths for instance. Certainly the results of cochlear microphonic threshold measurements taken from cats 1 3 and 4 tend to support the frequency maps Cat 1 for instance showed a cochlear microphonic threshold elevation at 4 kHz of 37 dB from the mean threshold at 1 2 and 3 kHz, and the elevation increased to more than 40 dB for frequencies of 8 kHz and above This is the expected cochlear microphonic threshold elevation pattern in view of the finding that outer hair cells are the most influential in cochlear microphonic production, exceeding that of inner hair cells by about 40 dB (Dallos & Wang 1974) Similar agreement between the pattern of hair cell losses and cochlear microphonic threshold elevations were found for cats 3 and 4

If cochlear frequency sharpening is reflected in both behavioural critical bandwidths and the bandwidth of neural tuning curves, then broadened critical bands should be expected under conditions of selective outer hair cell elimination in the same way as neural curves appear to be broadened as reported by Kiang et al. (1970) and Evans (1975*a* *b*) This expectation was not supported by the present experiment Instead normal critical band widths depended only upon the integrity of more than 40% of the population of inner hair cells in the relevant cochlear region It appears that selective outer hair cell elimination does not affect this frequency analytic function and that inner hair cells alone can account for cochlear frequency sharpening at least for the moderate stimulus intensity levels employed in this experiment.

These results may be accounted for by a model of inner–outer hair cell interaction proposed by Dallos et al. (1977) The model derives from tuning curves measured in normal and kanamycin-treated chinchillas The authors describe an outer hair cell contribution to tuning curves which is schematized as a flat-topped filter providing a frequency dependent gain on inner hair cell activity whose effect is greatest at and immediately surrounding the characteristic frequency Thus when outer hair cells are selectively eliminated the tuning curve tip is truncated but its bandwidth is not widened Cochlear frequency selectivity can therefore be mediated by inner hair cell activity alone while outer hair cells allow the inner hair cells to function at low signal levels around the characteristic frequency Accordingly Russell & Sellick (1977) have recorded intracellularly from inner hair cells in the guinea pig cochlea, and found that inner hair cells are tuned as sharply as auditory nerve fibres

Finally since it was found that critical bandwidths were not affected until at least 40% of inner hair cells were missing there appears to be a considerable redundancy in the inner hair cell system

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ZUSAMMENFASSUNG

Kritische Bandweiten und absolute Intensitätsschwellwerte wurden bei Katzen vor und nach Behandlung mit Kanamyzin gemessen, welche Verlust von inneren und äußeren Haarzellen bewirkte. Die Verluste von Haarzellen wurden an Cochleogrammen gemessen, die von Oberflächenpräparaten für das Organ von Corti konstruiert worden. Die Resultate lassen darauf schließen, daß für die Versuchsfrequenzen und Reizintensitäten, die benutzt wurden, kritische Bandweiten für Frequenzen, die tonotopisch zu Zonen der Cochlea lokalisiert waren, wo nur äußere Haarzellen verloren wurden, nicht beeinflußt wurden. Die kritischen Bandweiten wurden nur dann verbreitert oder nicht meßbar wenn Verluste von inneren Haarzellen, die 40% übersteigen auch vom kompletten Verlust von äußeren Haarzellen begleitet waren. Das Experiment läßt darauf schließen, daß die cochleare Frequenzselektivität von den inneren Haarzellen allein beeinflußt werden kann

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EFFECTS OF KANAMYCIN ON THE AUDITORY EVOKED RESPONSES DURING POSTNATAL DEVELOPMENT OF THE HEARING OF THE RAT

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Abstract The ototoxic effects of kanamycin were studied in rats during the early postnatal period and at an adult age. Brain stem potentials as well as auditory cortical potentials were used for the estimating of ototoxic damage The auditory potentials decreased promptly and markedly in the animals which were treated daily with 400 mg/kg body weight of kanamycin starting from the 11th day after birth In these animals, the auditory potentials were almost completely abolished within 10 days after the beginning of the kanamycin treatment However when the same amount of kanamycin was applied earlier or later than that, avoiding the period of the initial appearance and the greatest development of auditory functions (from the 11th to the 15th day after birth in the rat) the auditory potentials were not apparently damaged. In light and scanning electronmicroscopy marked ototoxic changes were observed which underlay the functional damage The meaning of these findings is discussed

The ototoxic effects of aminoglycosidic antibiotics have been intensely studied since the initial clinical reports on streptomycin appeared (Hinshaw & Feldman 1945 Brown & Hinshaw 1946) It has been established in both clinical and experimental studies that aminoglycosidic antibiotics suppress cochlear and vestibular functions by primarily damaging sensory hair cells (Hawkins 1959 Jorgensen & Schmidt 1962 Kohonen & Tarkkanen 1969) It is also well known that the ototoxic effects of aminoglycosidic antibiotics usually appear very slowly following systemic administration of the drugs in the adult animals (Hawkins, 1959 Aran and Darrouzet 1975 Farkashidy et al 1963) However little information is available on the susceptibility of auditory organs during their maturation process, since most of the investigations on the ototoxicity have been conducted on the adult animals

In the present study, rats in the early post natal period were treated with kanamycin and the damage thereby produced in hearing was studied by recording auditory evoked responses (Jewett & Romano 1972 Tokimoto et al. 1977) Rats were used because their auditory function as in mice is very poorly developed at birth (Kikuchi & Hilding, 1965) In some of the animals morphological studies of the sensory epithelium of the inner ear were studied with light and scanning electron microscopy

It was found in the present study that there is a specially susceptible period (critical period) which lasted for about a week in the early post-natal period. The mechanism underlying this finding was discussed from a point of view that the slow appearance of the ototoxic effects in adult animals may be attributable to their slow accumulation in the endolymphatic space (Stupp et al 1966 Voldrich 1965 Watanabe et al 1969 Toyoda & Tachibana, 1978)

MATERIAL AND METHODS

More than 105 rats from 18 different litters were used at various ages from birth to adulthood Recording electrodes were mounted on the scalp of each animal before they were used for the experiment. The operation was carried out under anesthesia with sodium pentobarbitone (30 mg/kg intraperitoneal) following a pre application of ether The small incision was made to the skin and some muscles were removed before two small pin-type recording electrodes were pricked into the skull They

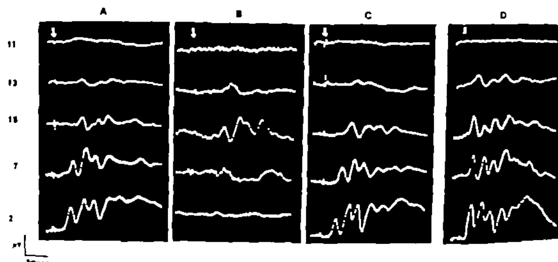


Fig 1 Sample records of BSRs indicating effect of kanamycin on hearing in neonatal rats Five responses in each column were obtained from one and the same rat on different dates Kanamycin (400 mg/kg) was applied from 1st to 10th day after birth (A) from 11th to 20th (B) and from 15th to 23rd (C) (D) Control records from a rat without administration of kanamycin. Figures on the left indicate the days after birth. Stimulus single wave of sound at 4 000 Hz and 80 dB SPL.

were fixed to the place with the help of dental cement or aron alpha The electrodes once mounted could usually be used throughout the course of the experiment When the electrodes were taken off they were refixed to the original site as far as possible

Kanamycin (daily dose of 400 mg/kg 200 mg/kg or less) was applied for certain days according to the schedule of the experiment In animals which received a daily dose of 400 mg/kg the increase in the body weight was slowed down as compared with the control Some of the animals especially younger ones died during the course of experiments but they were less than 10% in number None of the rats which received a daily dose of 200 mg/kg or less died In some animals gentamycin (150 mg/kg) and dehydrokanamycin B (DKB 200 mg/kg) were also applied

Summated auditory responses were recorded under light anesthesia (25 mg/kg pentobarbitone or ether only) at an interval of a few days Younger rats mostly recovered completely from the anesthesia within 4 or 5 hours and began to suckle If they did not begin to suckle several hours after the recording the data were discarded Data on adult animals were also discarded when they wakened too slowly after anesthesia in recording session.

Methods for recording have been described elsewhere (Tokimoto et al 1977) Potentials were averaged with an averaging computer (Nihon Kohden ATAC 201) 999 responses for the brainstem responses and 100 responses for the cortical evoked potentials The analysis time used was 10 msec for the brain stem responses (BSR) and 100–250 msec for the auditory evoked cortical responses (AEP) The output of the averaging computer was recorded by a camera or X–Y recorder (TOA XYR 1A) Electroencephalogram was monitored on an oscilloscope (Tektronix 5130N)

For sound stimulation tone pips or one cycle of sound wave of various intensities and frequencies were delivered through a loud speaker placed about 20 cm from the animal The rate of stimulation was about 1/sec for the AEP and 5–10/sec for the BSR The sound intensity was measured with a condenser microphone placed in between two ears and expressed with reference to the sound pressure level (0 0002 dyne/cm²) In some of the experiments simulated AEPs were evoked by delivering electrical shocks to the eighth nerve fibers within the cochlea For the stimulation a pair of tungsten wire electrodes (tip dia-

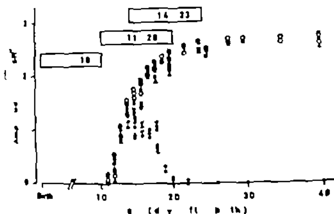


Fig. 2. Amplitude of BSRs plotted against animals' ages. Summary of results from three groups of rats treated with kanamycin (400 mg/kg daily for 10 days) with different time schedules. Periods of drug administration are shown together with symbols.

meter 0.1 mm, interpolar distance about 0.3 mm) were inserted into the cochlea near its apex.

For microscopy cochleas on either side were excised under ether anesthesia. Immediately after the excision cochleas were washed several times and fixed with 2.5% glutaraldehyde. Post fixation of the tissue was performed in buffered 1% OsO. Further processing of the specimen was done by alcohol dehydration and one cochlea was used for the scanning electron microscopy and the other for light microscopy. For the former critical point drying and sputtering with thin gold layer was made and the specimen was examined with an electronmicroscope (JSM 50A).

RESULTS

Effects on BSR of kanamycin administered at different stages of development

It was found in the present study that the effect of kanamycin on the BSR in the early neonatal period was quite different depending on the schedules of its administration. In the present series of experiment kanamycin was administered to neonatal rats with three different schedules. Typical results from one such experiment are illustrated in Fig. 1.

In normal rats not treated with kanamycin the BSR usually appeared first from the 11th to the 13th day after birth and showed a rapid development thereafter as shown in Fig. 1D (Jewett & Romano 1972, Tokimoto et al

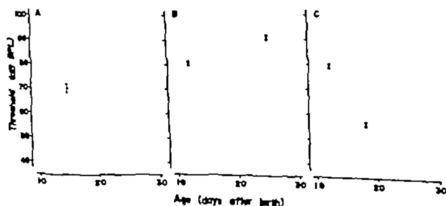


Fig. 3. Threshold sound intensity for BSRs in rats treated with kanamycin (400 mg/kg daily for 10 days) with three different time schedules. Kanamycin was applied from 1st ... 11th to 20th (B) and from 15th to 23rd (C). Open circles in (A) denote control obtained from the normal rats. Sound, 4000 Hz and 80 dB SPL.

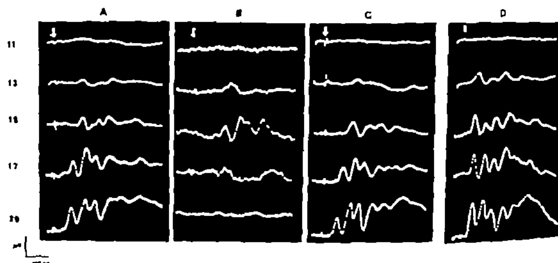


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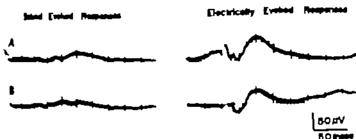


Fig. 5. Sound-evoked cortical responses (*AEP*) and electrically stimulated responses recorded from 2 rat treated with kanamycin. Kanamycin was applied from 11th to 20th day 400 mg/kg in (A) and 200 mg/kg in (B). Potentials were recorded on 23rd day after birth in (A) and on 25th day in (B).

depressed the auditory responses when applied from the 11th to the 20th day after birth. Namely the most effective actions were observed when these antibiotics were applied during the period which includes the phase of greatest development of the hearing.

Effects of kanamycin on the threshold sound intensity required for the production of the BSR

In this series of experiment the effect of kanamycin was measured by observing changes in the threshold sound intensity required just to evoke the BSR in neonatal rats. Sound frequency was 8000 Hz. Kanamycin (400 mg/kg) was applied daily for 10 days to 13 rats with three different schedules as in the previous series of experiment. Generally speaking, results obtained with this method were more or less similar to those obtained with the constant sound intensity.

In normal rats the threshold of the auditory responses decreased with the lapse of day following their initial appearance and became below 50 dB SPL within about 3 weeks after the birth. Now in a group of rats to which the drug application continued until just before the first appearance of the auditory response the time course of changes in the threshold was about the same as that observed in the control rats (Fig. 3 A). It was observed however that the reduction in the threshold took place less rapidly and also insufficiently as compared with the control. The final threshold was 5 to 20 dB higher than the control. But such differences were no more observed when the drug application was stopped 4 or 5 days prior to the first appearance of the BSR (open marks in Fig. 3 A).

On the contrary in a group of rats to which the same dose of kanamycin (400 mg/kg) was applied from the 11th to the 20th day after birth the threshold which had so far been decreasing began to increase after 4–5 days following the start of the drug application. The threshold continued to increase even after the end of the drug application eventually leading to a state in which no BSR was evoked even with the maximum intensity of the stimulus (110 dB SPL. Fig. 3 B).

When the administration of kanamycin (400 mg/kg) started about 2 weeks after the birth no detectable effect was produced on the time course of changes in the threshold (Fig. 3 C).

Effects of kanamycin on auditory responses of the adult rats

As a control experiment effects of kanamycin (400 mg/kg daily) on the BSRs were observed in more than 15 adult rats. All the rats used were more than 2 months old when the drug administration started. Fig. 4 A shows sample responses from a same rat. Stimulus sound used was 4000 Hz and 80 dB SPL. In Fig. 4 B normalized amplitude of the BSRs of all the animals tested are plotted against the number of days following the start of the drug administration. In normalizing the amplitude the average value of more than three BSRs obtained from each rat prior to the start of the drug application was taken as the norm and expressed as 100%. Responses recorded without the drug application usually fell within

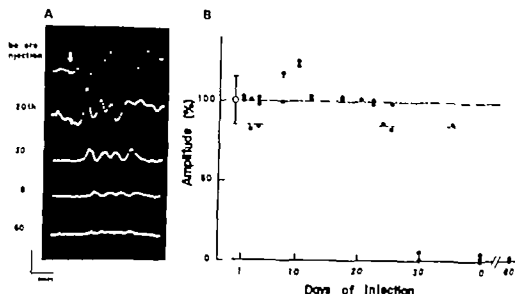


Fig 4 Changes in amplitude of the BSRs in adult rat treated with kanamycin with a daily dose of 400 mg/kg (A) Sample records showing development of drug effect in one and the same rat during drug application Attached figures denote days after starting drug application (B) Summary of results obtained from 16 rats See text for further explanations.

1977) Now in a group of rats kanamycin (400 mg/kg) was daily applied from the first day of the birth to the 10th day However as shown in Fig 1A the pattern development of the BSR in this group of rats was not much different from that observed in the con trol rat Results obtained in 7 other rats in this group are plotted with filled triangles in Fig 2 The only difference in this group of rats was a slightly smaller size of BSR as compared with the control group (open circles) But even this small difference in the amplitude was no more apparent when kanamycin was adminis tered only up to the 8th day namely when the administration of the drug was stopped 3–4 days before the initial appearance of the BSR

On the contrary a marked suppression of the BSRs was observed in the group of rats to which kanamycin (400 mg/kg) was administered from the 11th to the 20th day after birth As shown in Fig 1B the BSRs in this group of rats developed apparently normally up to the 15th day or so but responses tended to decrease rapidly thereafter and were almost abolished after the 20th day More than 15 rats were treated with kanamycin with this schedule and the results obtained were plotted in Fig 2 with filled circles Without any exception the BSRs in this group of rats began to decline markedly after the 15th day of the birth i e the fourth day following the start of the drug administration and the BSRs were almost totally abolished after the 20th day of the birth Also almost no recovery was observed after the end of the drug administration With this schedule of administration kanamycin at a daily dose of 200 mg/kg produced a quite marked effect on the BSR Namely the amplitude of the BSR was depressed in the average below 30% of the control

When the start of the drug application was delayed slightly i e until after a few days following the start of the initial rapid phase of development of the BSR the application of the same daily dose of kanamycin (400 mg/kg) for 10 days produced almost no effect on the BSRs (Fig 1C) Results obtained from 7 rats of this group are plotted in Fig 2 with filled squares

Effects of gentamicin and DKB on BSR and AEP were similar to those of kanamycin Gentamycin and DKB with a daily dose of 150 and 200 mg/kg respectively almost completely

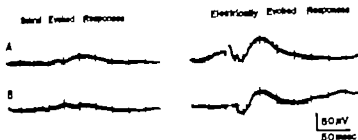


Fig. 5. Sound-evoked cortical responses (*AEP*) and electrically stimulated responses recorded from 2 rats treated with kanamycin. Kanamycin was applied from 11th to 20th day 400 mg/kg in (A) and 200 mg/kg in (B). Potentials were recorded on 23rd day after birth in (A) and on 25th day in (B).

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Effects of kanamycin on the threshold sound intensity required for the production of the BSR

In this series of experiment, the effect of kanamycin was measured by observing changes in the threshold sound intensity required just to evoke the BSR in neonatal rats. Sound frequency was 8000 Hz. Kanamycin (400 mg/kg) was applied daily for 10 days to 13 rats with three different schedules as in the previous series of experiment. Generally speaking, results obtained with this method were more or less similar to those obtained with the constant sound intensity.

In normal rats, the threshold of the auditory responses decreased with the lapse of day following their initial appearance and became below 50 dB SPL within about 3 weeks after the birth. Now, in a group of rats to which the drug application continued until just before the first appearance of the auditory response, the time course of changes in the threshold was about the same as that observed in the control rats (Fig. 3 A). It was observed, however, that the reduction in the threshold took place less rapidly and also insufficiently as compared with the control. The final threshold was 5 to 20 dB higher than the control. But such differences were no more observed when the drug application was stopped 4 or 5 days prior to the first appearance of the BSR (open marks in Fig. 3 A).

On the contrary, in a group of rats to which the same dose of kanamycin (400 mg/kg) was applied from the 11th to the 20th day after birth, the threshold, which had so far been decreasing, began to increase after 4–5 days following the start of the drug application. The threshold continued to increase even after the end of the drug application, eventually leading to a state in which no BSR was evoked even with the maximum intensity of the stimulus (110 dB SPL, Fig. 3 B).

When the administration of kanamycin (400 mg/kg) started about 2 weeks after the birth, no detectable effect was produced on the time course of changes in the threshold (Fig. 3 C).

Effects of kanamycin on auditory responses of the adult rats

As a control experiment, effects of kanamycin (400 mg/kg, daily) on the BSRs were observed in more than 15 adult rats. All the rats used were more than 2 months old when the drug administration started. Fig. 4 A shows sample responses from a same rat. Stimulus sound used was 4000 Hz and 80 dB SPL. In Fig. 4 B normalized amplitude of the BSRs of all the animals tested are plotted against the number of days following the start of the drug administration. In normalizing the amplitude, the average value of more than three BSRs obtained from each rat prior to the start of the drug application was taken as the norm and expressed as 100%. Responses recorded without the drug application usually fell within

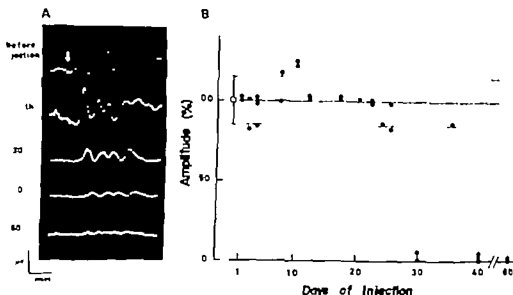


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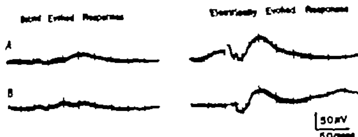


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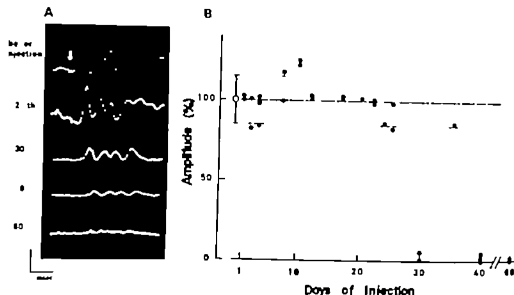


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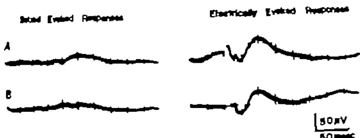


Fig 3 Sound-evoked cortical responses (*AEP*) and electrically stimulated responses recorded from rats treated with kanamycin. Kanamycin was applied from 11th to 20th day 400 mg/kg in (A) and 200 mg/kg in (B) Potentials were recorded on 23rd day after birth in (A) and on 25th day in (B)

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A
B
C
D

+15% of the norm as shown with broken lines in Fig 4B

Effects of kanamycin in adult rats appeared very slowly The decrease in the amplitude of the BSRs took place only after 3 weeks follow ing the start of the drug application Thereafter however reduction in the BSR s amplitude progressed more or less quickly leading to their total abolishion In some animals the depression produced by kanamycin was preceded by a slight enhancement of the re sponse (15–40%) Similar enhancement has been observed in the compound eighth nerve action potential of the guinea pig (Aran & Derrouzet, 1975) and in the microphonic potentials of the goldfish saccule (Matsuura et al 1971). Usually no change in the response amplitude was observed prior to the end of the first week of kanamycin treatment

Electrically simulated cortical and tory responses in animals treated with kanamycin

It has been demonstrated that the hair cells were easily damaged by aminoglycosidic antibiotics, but nerve fibers and their endings showed a much greater resistance (Duvall & Wersäll 1964 Farkashidy et al 1963) Kiang et al (1976) have reported that many cochlear afferent fibers in kanamycin-treated animals were found responsive to electrical stimulation, though they were unresponsive to sound In the present study the intactness of the cochlear afferent fibers was demonstrated by de livering single electrical stimulation to the cochlea and by observing the cortical responses evoked by the stimulation. The stimulus was applied through a bipolar electrode inserted into the modiolus. Kanamycin (400 and 700 mg/kg) was applied to two groups of rats for 10 days from the 11th to the 20th day after birth As shown in Fig 5 left auditory evoked cortical responses in these rats were very small in amplitude and evoked with a longer latency Stimulus sound used was 1000 Hz and 90 dB SPL. But, the simulated cortical responses evoked by elec trical stimulation of the auditory nerve were not different from those observed in normal animals (Fig. 5 right) The stimulus intensity necessary to evoke maximum cortical re sponses in these treated animals stayed more or less in the same range as in normal rats

Morphological changes produced by kanamycin of hair cells in various developmental stages

Fig 6A shows what the normal sensory epithelium of the 18-day-old rat looks like As has been reported by many investigators (Hawkins, 1959 Bosher & Warren 1971 Mikaelian & Ruben 1965 Kikuchi & Hilding 1965) the organ of Corti of the rat develops almost fully within 3 weeks after birth When kanamycin sulfate (400 mg/kg) was applied for 4 days starting from the 11th day after birth and the animals were sacrificed on the follow ing day evident damage was detected in the organ of Corti, as shown in Fig. 6B C In these pictures a sporadic loss of sensory hairs was observed along with the fusion of hairs which was more frequent. Various other changes indicating a moderate injury to the sensory hairs existed but the degree of cellular damage was not uniform among animals treated in the same way When kanamycin was administered for a longer period i e for 7–10 days starting from the 11th day after birth pronounced damage was inflicted on almost every outer hair cell in every turn There was a near-total or complete loss of the outer hair cells in the basal turn while the inner hair cells seemed to be well preserved (Fig 6D) When kanamycin was applied before or after the period of the greatest development of hearing, the organ of Corti showed almost no change after treatment with the drug for 7–10 days Thus a clear correlation was found be tween the functional losses as demonstrated in

Fig 6 Scanning electronmicroscopic pictures of the sensory hairs. (A) Normal rat 18 days old. (B) rat 15 days old to which kanamycin was applied from 11th to 14th day after birth. (C) same as (B), but obtained from different rat; (D) rat 29 days old to which kanamycin was applied from 11th to 18th day after birth Calibration, 2 μm.

the BSRs and the damage to the hair cells as revealed by scanning electronmicroscopy

DISCUSSION

Aran & Darrouzet (1975) reported that click evoked VIII nerve responses in the guinea pig recorded with implanted electrodes decreased very markedly in amplitude within 8–10 days when treated with kanamycin for 8 days on a 400 mg/kg basis while in the present experiment auditory evoked responses of the adult rats (older than 60 days) showed almost no sign of decrease until 20 days even when the same daily doses of kanamycin were applied (Fig 4) Thus the result of the present study indicates that rats are less susceptible to kanamycin than cats and guinea pigs in agreement with an earlier report by Hawkins (1959)

The main purpose of the present study i e to demonstrate the presence of a specially susceptible period to kanamycin was fairly well attained When kanamycin (400 mg/kg to 200 mg/kg) was applied daily from the 11th day after birth the BSR began to decrease within 4 days and almost disappeared within 10 days The depressive action was quite drastic and different from those observed at other stages of development namely a daily administration of kanamycin (400 mg/kg) for 7–10 days barely modified the BSR when delivered preceding ro following that special period

The critical period observed in connection with the special susceptibility to kanamycin seems to coincide with the period of very rapid development in terms of the morphology and physiology of the inner ear Bosher & Warren (1971) reported that the endocochlear potential and the cochlear microphonic potential in rats showed a sudden increase in size during the short period extending from the 11th to the 15th day after birth The same thing was observed with the BSR (Tokimoto et al 1977) At the beginning of the above period the stria vascularis tectorial membrane and the organ of Corti including the hair cells supporting cells and the tunnel of Corti are completely differentiated In particular the scala media and the perilymphatic scalae are fully formed at the period but there remains some rudimentary cells of Claudius lining the external sulcus (Bosher & Warren 1971) Similar findings were reported by Mikaelian & Ruben (1965) in the normal CB-J mouse Kikuchi & Hilding (1965) also reported that in normal mice the organ of Corti appeared fully mature with stria vascularis almost complete at the time when cochlear microphonic potentials could be first recorded.

In this connection it is tempting to speculate on the mechanism which underlies the specially susceptible period to the kanamycin. It is now generally accepted that aminoglycosidic antibiotics affect primarily the organ of Corti especially the hair cells (Duvall & Wersäll 1964 Hawkins & Engström 1964 Kohonen & Tarkkanen 1969) although damage to stria vascularis plexus cochlearis and supporting cells has also been reported (Mootz et al 1972 Reddy & Igarashi 1962) One hypothesis by which to explain the presence of the critical period is that the antibiotics might gain access more easily during the critical period to the site of action on hair cells. Although direct evidence allowing one to localize the site of action of the antibiotics on the hair cells are still lacking a very prompt and reversible depression which appeared immediately after a local application to the excised lateral line and ampullar tissues as well as the decline in the microphonic potentials of the goldfish saccule upon intraluminal application may suggest a direct interference with the receptor mechanism acting on rather superficial structures presumably located on the hair-bearing surface of the sensory hair cells. At least initially their bonds with the substratum do not seem to be deep (Harada et al, 1967 Wersäll & Flock 1964 Matsuura et al 1971 Duvall & Wersäll 1964 Hawkins & Engström 1964 Kohonen 1965 Wersäll et al 1973 Stockhorst & Schacht 1977 Theopold 1977) Therefore the ve slow initiation

of the ototoxic action of the antibiotics upon repeated systemic application and no occurrence of the ototoxic action in their single application in adult animals may be ascribed to a slow accumulation of the drugs in the inner ear and their protracted elimination (Vordlich 1965 Toyoda & Tachibana 1978 Mootz et al., 1972, Tachibana et al 1978) and also to the difficulty in gaining access to the topical site of action on the hair cells from the perilymphatic spaces (Harada et al 1967 Nuttall et al 1977)

In line with the above interpretation the rapid development of ototoxic effects of the antibiotics which appeared at the stages of the greatest development (from the 11th to the 15th day after birth) could mean that the drugs easily invade the endolymphatic space through structures such as the stria vascularis which has just started functioning or through the external sulcus which reaches full maturity only about 15 days after birth According to this explanation it would not be necessary to assume an especially elevated susceptibility on the part of hair cells vis-à-vis the antibiotics at a certain developmental stage Of course further investigation is necessary before the mechanism underlying the critical period can be more fully elucidated In particular the processes involved in the invasion and elimination of the antibiotics to and from the peri- and endolymphatic spaces must be clarified in greater detail (Tachibana et al 1978 Mootz et al. 1972 Nuttall et al 1977 Toyoda & Tachibana 1978)

However a finding especially relevant to the present study is the augmentation of the ototoxic effects by some diuretics Namely a much faster occurrence of ototoxic effects of systemically applied aminoglycosidic antibiotics was demonstrated in the adult guinea pigs in connection with the use of furosemide or ethacrinic acid. The effect appeared after the auditory responses had almost fully recovered from the depressive effect of the diuretics alone (Bosher et al 1973) when a single dose of antibiotic was applied combined with the diuretics (Brummett et al 1975 West et al 1973 Nakai 1977) Ethacrinic acid produces even with a single administration morphologically detectable transient damage to the stria vascularis (Quick & Duvall 1970) along with abnormalities in membrane permeability (Bosher et al 1973) Thus there seem to be many similarities between the critical period and the enhancement of the ototoxic effects by the diuretics It seems quite probable that the two phenomena may share a common factor i e a facilitated entry of the antibiotics into the endolymphatic space

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ZUSAMMENFASSUNG

Untersucht wurde die spezifisch ototoxische Wirkung des Kanamycins auf die auditiven Funktionen der Ratten während der verschiedenen Stufen der postnatalen Entwicklung und im erwachsenen Alter Zur Erfassung einer akustischen Schädigung wurden die *Hirnstammpotentiale* sowie die cortikalen Potentiale abgeleitet. Die akustischen Potentiale fielen in dramatischer Weise in den Tieren ab die mit Kanamycin (400 mg/kg Korpergewicht) vom *11ten Tage an nach Geburt täglich behandelt* wurden, und fast keine Potentiale wurden am 10ten Tage nach dem *Beginn der Kanamycin-Behandlung* beobachtet Wenn das Kanamycin (400 mg/kg) täglich für 10 Tage *abgegeben* wurde – *unter Vermeidung* der Periode der schnellsten und größten Entwicklungsstufe von auditiven *Funktionen der Ratten (am 11ten bis zum 15ten Tage* nach Geburt) – wurden die Potentiale kaum schädlich verändert Licht- und *rasterelektronenmikroskopisch* fanden wir entsprechend den Funktionsschädigungen, stark und bedeutend toxische *Veränderungen an den* äußeren Haarzellen nach der Kanamycin-Injektion (400 mg/kg) während der *schnellsten und größten* Entwicklungsperiode *Die* Bedeutung der Resultate wurde diskutiert

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OTOTOXIC EFFECTS OF THE INTERACTION BETWEEN KANAMYCIN AND ETHACRYNIC ACID

Cochlear Ultrastructure Correlated with Cochlear Potentials and Kanamycin Levels

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Abstract. The effects of the interaction between kanamycin (KAN) and ethacrynic acid (EA) on the ultrastructure of the guinea pig cochlea were studied 3 4 6 and 24 hours following administration of EA (40 mg/kg) to animals pretreated 2 h earlier with KAN (400 mg/kg). Appropriate saline (SAL) controls were included giving 4 treatment KAN/EA, KAN/SAL, SAL/EA and SAL/SAL. The outer hair cells of the organ of Corti showed nuclear and plasma membrane changes at 3 h and were completely destroyed by 4 h. The inner hair cells were unaffected. Severe swelling was seen in the stria vascularis of both KAN/EA and SAL/EA animals at 3 h and was gone by 24 h. KAN/EA had greater effect on the stria than had SAL/EA. These results were consistent with the time course of the effect of the drugs on the and d c. endocochlear potentials. KAN concentrations in perilymph were unaffected by treatment with EA

Permanent hearing losses are produced by aminoglycoside antibiotics such as streptomycin, neomycin kanamycin gentamicin and tobramycin. Destruction of sensory hair cells of the inner ear in man and in experimental animals has been found after prolonged treatment with large doses of these antibiotics (Hawkins et al. 1952, McGee & Olszewski 1953 Frost et al 1960; Ruben & Daly 1968 Webster et al 1970; Brummett et al 1971)

Rapid onset hearing losses of a transient nature are produced by another group of drugs the potent loop-inhibiting diuretics which include ethacrynic acid and furosemide (Ng et al 1969; Schwartz et al 1970; Brummett et al 1977) As the ototoxic effects of both groups of drugs became more widely known, clinical ... eared indicating that patients who had received simultaneous therapy with an aminoglycoside antibiotic and a loop-inhibiting diuretic suffered severe permanent hearing losses of rapid onset (Mathog & Klein 1969 Johnson & Hamilton 1970 Meriwether et al 1971) This result was unexpected since the doses of drugs from both groups were well below those known to cause ototoxicity when used singly It was concluded that the loop-inhibiting diuretics interacted with the aminoglycoside antibiotics to produce the augmented ototoxic effect.

The interaction of aminoglycoside antibiotics with the loop-inhibiting diuretics was subsequently confirmed in animal experiments Hair cell loss has been demonstrated in the guinea pig cochlea as a result of the interaction of kanamycin and ethacrynic acid (West et al 1973 and Prazma et al 1974) and kanamycin and furosemide (Brummett et al 1975) More recently the interaction of 3 4 -dideoxykanamycin with ethacrynic acid has been reported by Nakai (1977) In addition additive ototoxic effects of streptomycin and ethacrynic acid have been found in the feline vestibular system as a result of the interaction of these drugs (Mathog & Capps 1977) No interaction has been found between kanamycin and thiazides mercurials carbonic anhydrase inhibitors or osmotic diuretics (Brummett et al. 1974)

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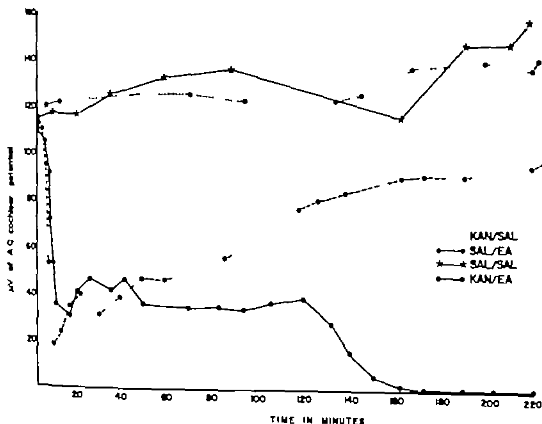


Fig 1 c. cochlear potentials from guinea pigs following the second injection of the indicated treatments (see Methods) The sound stimulus was at 7 kHz and of sufficient intensity (about 1 dyne/cm²) to produce 100 to 120 μV of cochlear potential (zero-time values) In the series of experiments shown the animals cochleas were perfused at 4 h for preparation for EM

Descriptions of the methods used in measuring the a.c cochlear potential including animal preparation sound pressure measurement, and radiation artifact elimination were reported earlier (Brummett et al. 1972 West et al. 1973) The d.c endocochlear potential was measured with a small microelectrode (tip diameter 2–3 μm) filled with synthetic endolymph (Bosher & Warren 1968) which was inserted into the scala media through the round window membrane Details of this method have been reported previously (Brummett et al 1977)

Kanamycin concentrations were measured in blood and perilymph of all animals prepared for EM study Concentrations of kanamycin were measured in both fluids by an enzymatic method described by Broughall & Reeves (1975) This method utilizes an aminoglycoside acetylating enzyme to acetylate kanamycin using radiolabeled coenzyme A. The assay method was sensitive to 0.5 μg/ml of kanamycin. Perilymph collection plasma preparation and their assay are described elsewhere (Brummett et al 1978)

Immediately after termination of the electrophysiological studies the electrodes were removed from the cochlea and the cochlea perfused *in situ* with Dalton s osmium-dichromate (Dalton, 1955) Next the bulla was opened to expose the cochlea and the cochlear tissues fixed further by immersion. The cochlea was thoroughly dehydrated then embedded *in toto* in Spurr low viscosity resin (Spurr

The present investigation followed the progression of ultrastructural changes induced in the cochlear tissues of the guinea pig as a result of the interaction of kanamycin and ethacrynic acid and correlated these changes with changes in cochlear function and kana mycin levels Changes in cochlear function were determined by monitoring the a c cochlear and d c endocochlear potentials At pre determined times during the course of the interaction cochlear tissues were examined by electron microscopy and kanamycin con centrations in plasma and perilymph deter mined

METHODS AND MATERIALS

A total of 22 young adult guinea pigs (300–350 g) with a positive Preyer pinna reflex were used in these experiments An electron microscopic (EM) study was made four times during the interaction between kanamycin and ethacrynic acid (3 4 6 and 24 h) Animals of the 3- 4- and 6-h series were anesthetized with intraperitoneal injection of Dial (60 mg/kg) with urethane (240 mg/kg) Baseline determinations were made of the ability of the left cochlea to generate an a.c cochlear potential Next a single 400 mg/kg dose of kanamycin or an equal volume of saline (0 15 M NaCl) was injected subcutaneously Two hours later sufficient sound (about 1 dyne/cm^2) at 7 kHz was introduce into the ear to generate 100 to 120 μV of a.c cochlear potential This was fol lowed by a single 40 mg/kg intravenous dose of ethacrynic acid or an equal volume of saline (0 07 M NaCl) given over a one minute period exactly 2 h after the initial injection of kana mycin or saline The time at which ethacrynic acid or its saline equivalent was injected is referred to as zero time The a c cochlear potential was monitored continuously throughout the experiment At specific times the d c endocochlear potential of the same cochlea was measured and that cochlea perfused with fixative for EM preparation Immediately following the perfusion blood was withdrawn from the left ventricle of the heart into a heparinized syringe and perilymph was tak from the right cochlea. The perilymph and t plasma prepared from the blood were assay for kanamycin

Animals of the 24-hour series were giv a single 400 mg/kg dose of kanamycin (saline) and then anesthetized with pentobarb tal (35 mg/kg i p) one hour later Anima were surgically prepared under sterile cond tions for recording the a c cochlear potenti and control measurements were then mad Two hours after the injection of kanamycin (saline) a single injection of 40 mg/kg of eth crynic acid (or saline) was given over o minute The a c cochlear potential was mor tored at 7 kHz for 18–20 min following t second injection Then the incisions we closed and the animals allowed to reco from the anesthetic Approximately 23 h lat the animals were anesthetized with the Dia urethane mixture and the a.c cochlear pote tial measured At 24 h the d c potential w measured the left cochlea perfused with fix tive and blood and perilymph taken as d scribed for the shorter time series

Thus at each of the four times chosen f our EM studies four different treatment re mens resulted kanamycin followed in 2 h b ethacrynic acid (KAN/EA) saline in place kanamycin followed in 2 h by ethacrynic ac (SAL/EA) kanamycin followed in 2 h b saline (KAN/SAL) and saline in place of bo drugs (SAL/SAL) The study was performe on a blind basis and the code broken only aft all data from each animal of a given series ha been fully evaluated

A separate series of experiments was pe formed on a group of 6 guinea pigs to measur the d c endocochlear potential from zero tim (time of the second injection) to 4 h Thes animals were treated with either KAN/EA KAN/SAL or SAL/EA and prepared in manner identical with the 3- 4- and 6-h studie described above In these studies however the d c endocochlear potentials rather tha the a.c cochlear potentials were monitore continuously

Table I. *d c endolymphatic potentials (mV) of guinea pigs obtained just prior to preparation for electron microscopic examination*

	Time after second injection (h)			
Treatment	3	4	6	4
KAN/SAL	6_	89	98	82
SAL/SAL	75	82	84	82
KAN/EA	67	28	13	38
SAL/EA	61	60	83	58

See Methods

guinea pigs were monitored continuously over a 4-h period (Fig. ?) During the first 20 min after KAN/EA or SAL/EA the d c endocochlear potential fell and recovered parallel with that of the a.c cochlear potential. Although the KAN/EA animals did not show a second drop in their d.c endocochlear potentials during this study the single d c measurements recorded on the KAN/EA animals of the 3 4 6 and 24 h series indicated that the d c levels fell just at or slightly later than 4 h after KAN/EA (Table I) These latter data show that at 3 h, when the a.c cochlear potentials were at very low levels the d c endocochlear potentials of the KAN/EA and SAL/EA animals were similar but by 4 and 6 h the d c endocochlear potentials of the KAN/EA animals were less than 30% of those of the SAL/EA animals and other controls Partial recovery of the d c potential of the KAN/EA animals was evident by ?4 h

Table II *Plasma and perilymph concentrations of kanamycin in guinea pigs obtained just prior to preparation for electron microscopic examination*

Time after second injection (h)	Treatment*	Kanamycin concentration (µg/ml)	
		Plasma	Perilymph
3	KAN/SAL	707	107
3	KAN/EA	1 607	97
4	KAN/SAL	283	7?
4	KAN/EA	987	173
6	KAN/SAL	190	63
6	KAN/EA	263	66
4	KAN/SAL	1 0	6.1
4	KAN/EA	2.1	7.3

See Methods

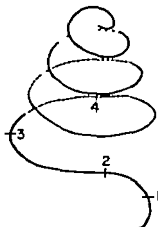


Fig 3 Specific sites in the basal end of the cochlea that were examined by electron microscopy for determination of ultrastructural changes in cochlear tissues.

Pharmacokinetics

The concentrations of kanamycin found in the plasma and in the perilymph of the right ear at the 3 4 6 and 24 h sampling times are shown in Table II At 3 6 and 24 h the perilymph kanamycin levels of the animals receiving KAN/EA were similar to those receiving KAN/SAL. On the other hand the KAN/EA animal in the 4 h series had a significantly higher perilymph kanamycin level than its KAN/SAL control Measurement of perilymph kanamycin levels in additional KAN/EA and KAN/SAL animals showed that similar high kanamycin levels occurred at 4 h after KAN/SAL. Thus there appears to be no difference between the level of kanamycin in the perilymph after KAN/EA and KAN/SAL. The plasma kanamycin concentrations after KAN/EA were consistently higher than those after KAN/SAL by a factor of about 2.

Ultrastructure

Electron microscopic studies were made at four specific sites at the basal end of the cochlea (Fig. 3) The ultrastructural changes de

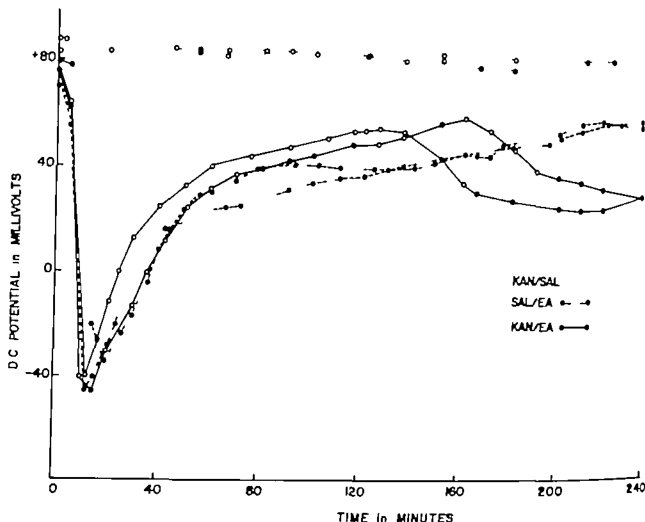


Fig 2 d.c endocochlear potentials from guinea pigs following the second injection of the indicated treatments (see Methods) Results are shown for two animals in each treatment group

1969) Four areas of the basal turn of each cochlea were prepared for study of the stria and organ of Corti (Fig 3) Thin sections of 500–900 Å were cut with a diamond knife on a Porter Blum MT 2B ultramicrotome placed on uncoated copper grids stained with uranyl acetate (Watson 1958) and lead citrate (Venable & Coggeshall 1965) and viewed with a Philips 300 electron microscope

RESULTS

All animals except those in the 24-hour series were monitored continuously throughout the the experimental period so that the patterns of changes in the a c cochlear potential output following drug administration could be determined Animals receiving identical drug treatments had patterns similar to those shown for the 4-h series in Fig. 1 KAN/SAL or SAL/SAL animals exhibited no change in their a c cochlear potential during the course of the experiments However after SAL/EA or KAN/EA there was an immediate fall in the a c cochlear potential that reached a minimum within 10–20 min This was followed by a period of gradual recovery during the next 1½ h In the SAL/EA animals recovery continued until pre-drug a c potential levels were reached However in KAN/EA animals there was a second fall in the a c cochlear potential at about 2 h that reached zero by 3–4 h

To determine if the a c cochlear potential changes were accompanied by changes in the d c endocochlear potential the d c endocochlear potentials of a separate group of 6

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4	KAN/SAL	1 0	6 1
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See Methods.

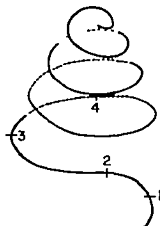


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animals and other controls. Partial recovery of the d c potential of the KAN/EA animals was evident by 24 h

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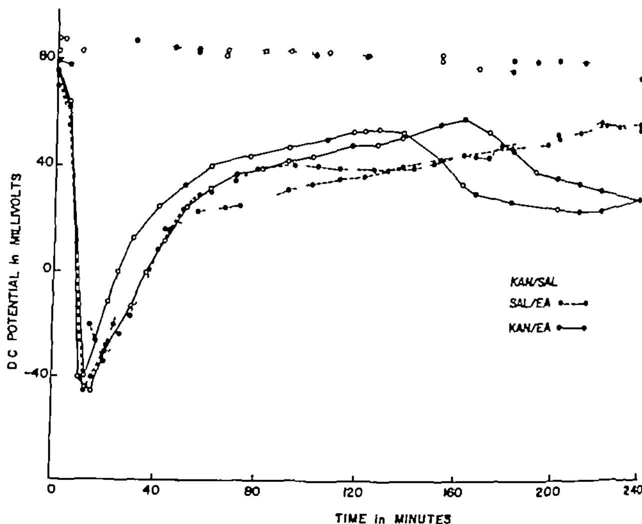


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scribed below were similar at each of the four sites within a given cochlea unless specifically noted.

Stria vascularis

The ultrastructure of the stria vascularis of the SAL/SAL controls (Fig. 4 A) was similar to that described by Hinojosa & Rodriguez Echandia (1966) in their ultrastructural studies of the cat cochlea. The stria vascularis of the SAL/SAL animals measured 25 μm basal cell to marginal cell and was composed of cells tightly apposed to one another (Fig 4 A) Within the cytoplasm of the marginal cells were numerous small vesicles (0 10–0.25 μm diameter). Following treatment with SAL/EA (Fig. 4B) or KAN/EA (Fig 4 C) but not after KAN/SAL, accumulations of intercellular fluid were seen within the stria vascularis. Although both SAL/EA and KAN/EA caused the stria to swell the extent and time courses of the swelling differed

After SAL/EA the stria showed moderate but variable swelling at 3 h characterized by enlarged intercellular spaces (Fig. 4B) By 4 and 6 h after SAL/EA the swelling was less pronounced and by 24 h few signs of edema remained. At 3 4 and 6 h after SAL/EA numerous large vesicles of up to 3 μm in diameter were found in the cytoplasm of the marginal cells (Fig. 4B) but at 24 h few were found

Fig. 4 Electron micrographs of stria vascularis of the basal turn 3 h after drug treatment (A) SAL/SAL control The stria is composed of three layers of closely apposed cells The marginal cells, *m* which border the scala media, *sm*, contain numerous small vesicles Along the lower border and attached to the spiral ligament, *sl* are the basal cells, *b* Between the basal and marginal cells there are the intermediate cells, and small blood vessels 400 (B) 3 h after treatment with ethacrynic acid alone (SAL/EA), the stria is edematous Large intercellular spaces are found particularly around the intermediate cells Marginal cells contain numerous vacuoles (arrow) 2400 (C) 3 h after KAN/EA the stria vascularis is even more swollen than after SAL/EA Large intercellular spaces are present around intermediate cells and between cells of the basal and marginal layers extending to the inner end of their tight junctions Strial thickness is nearly twice that of the SAL/SAL controls 400

Strial swelling 3 h after KAN/EA was greater and less variable (Fig. 4C) than that seen 3 h after SAL/EA Swelling was less at 4 and 6 h after KAN/EA but still greater than that seen in the 4 and 6 h SAL/EA animals The tight junctions of the marginal and basal cell layers appeared to be intact even in the severely swollen stria of the 3 h KAN/EA animal As a result fluid accumulated around the intermediate cells and capillaries separating them from the marginal and basal cells until only a lacy network of protoplasmic processes connected them. The luminal surfaces of the swollen stria vascularis were convex and bulged into the endolymphatic space The stria vascularis of the 6 h KAN/EA animal showed some areas with large fluid accumulations between the basal and intermediate cells and other areas which exhibited only moderate edema (Fig. 5) At 24 h little if any swelling remained. In fact 24 h after KAN/EA the stria vascularis was thinner than the SAL/SAL controls and contained some cell debris and degenerating cells suggesting that the earlier severe swelling resulted in significant cell death (Fig 6)

Hair cells

Inner hair cells (IHCs) and outer hair cells (OHCs) were normal in appearance after SAL/SAL (Fig 7 A) and KAN/SAL. Minor changes were seen in the OHCs after treatment with SAL/EA but these were not evident until 24 h after drug treatment, at which time the OHCs of the basal turn were slightly swollen at the level of the nucleus

Significant damage was seen in the OHCs as a result of the interaction between kanamycin and ethacrynic acid In the 3 h KAN/EA animal OHCs in the area of the round window membrane were swollen and their nuclei had become round and the chromatin clumped. Higher in the basal turn (Site 4 Fig. 3) the hair cells were still elongated (Fig. 7B) but the nuclei had lost their oval shape and instead were rounder with an irregular nuclear envelope and clumped chromatin In addition


b
k
s m
m
s l
4A


4B


4C

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Significant damage was seen in the OHCs as a result of the interaction between kanamycin and ethacrynic acid In the 3 h KAN/EA animal, OHCs in the area of the round window membrane were swollen and their nuclei had become round and the chromatin clumped Higher in the basal turn (Site 4 Fig. 3) the hair cells were still elongated (Fig. 7 B) but the nuclei had lost their oval shape and instead were rounder with an irregular nuclear envelope and clumped chromatin In addition

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Fig 4 Electron micrographs of stria vascularis of the basal turn 3 h after drug treatment (A) SAL/SAL control The stria is composed of three layers of closely apposed cells The marginal cells, *m* which border the scala media, *sm*, contain numerous small vesicles Along the lower border and attached to the spiral ligament, *sl* are the basal cells, *b* Between the basal and marginal cells there are the intermediate cells and small blood vessels. ×2400 (B) 3 h after treatment with ethacrynic acid alone (SAL/EA), the stria is edematous Large intercellular spaces are found particularly around the intermediate cells. Marginal cells contain numerous vacuoles (arrow). ×2400 (C) 3 h after KAN/EA the stria vascularis is even more swollen than after SAL/EA. Large intercellular spaces are present around intermediate cells and between cells of the basal and marginal layers extending to the inner end of their tight junctions Strial thickness is nearly twice that of the SAL/SAL controls ×2400



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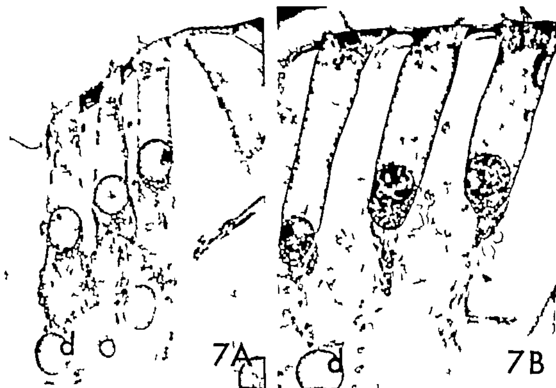


Fig. 7. Outer hair cells of the basal turn 3 h after treatment. (A) SAL/SAL control. Hair cells are elongated and nuclei are similar in appearance to those of the supporting Deiters cells, *d* beneath. 1800. (B) 3 h after KAN/EA. Hair cells of the upper region of the basal turn (site 4) are still intact but the nucleus has a crenated appearance and contains clumped chromatin. Nuclei of the Deiters cells *d* look normal. 1800.

were severely swollen and contained swollen nuclei. The stereocilia of these cells were also severely altered. Most of the cilia had a fused appearance due to the plasma membrane having lifted partially off the fibrous cores in a sheet (Fig. 10). Few lateral subsurface cisternae were found along the rounded surfaces of the swollen OHCs and some of these cisternae were dilated. Mitochondria that normally lie in close proximity to the lateral cisternae were either swollen or absent. In addition, few free ribosomes and little cytoplasmic ground substance remained in these swollen cells.

Twenty four h after KAN/EA the OHCs, including their cuticular plate, had disappeared from all three rows. Concomitant with the loss of these cells, the pillar cells had become distorted and the tunnel filled in by the adjacent cells (Fig. 11). The Nuel spaces and areas originally occupied by the OHCs had been filled in by Deiters and Hensen's cells. Phalangeal processes of the Dieters cells filled in spaces in the reticular lamina. Occasionally openings in the reticular lamina were seen.

The ultrastructure of the IHCs of the basal turn was normal in all KAN/EA animals even though all of the OHCs showed marked evidence of cell damage at 6 h (Fig. 9). Likewise the IHCs were normal in appearance at 24 h while the OHCs had been completely destroyed.

DISCUSSION

We have examined the ultrastructure of cochlear tissues 3, 4, 6 and 24 h after combined





6

Fig 5 Stria vascularis 6 h after KAN/EA Swelling is highly variable due to some reduction in fluid between the intermediate and marginal cells. The largest accumulations of fl id are seen the basal portion of the stria near the spiral ligament × 400

Fig 6 Stria vascularis 4 h after KAN/EA. The asculari no longer edematous but is thinner normal probably as result of cell death, as sugge by the damaged cell show here × 400.

the plasma membranes at the apical ends of the OHCs and stereocilia were deeply crinkled and in some areas the lateral subsurface cisternae that normally lie in layers along the lateral plasma membrane of the OHCs were missing (Fig 8)

At 4 and 6 h post-drug all three rows of OHCs in the basal turn of the KAN/EA animal were damaged (Fig 9) Frequently all that was left of the innermost outer hair cell was t apical end with the cuticular plate containin few fibrous cores and dense rootlets of t cilia. Junctional complexes were still prese between the Deiters cells and these apical ce remnants Cells of the middle and outermo rows of OHCs were generally identifiable bu grossly distorted with little remaining of th innermost OHC Many hair cells seen at 6

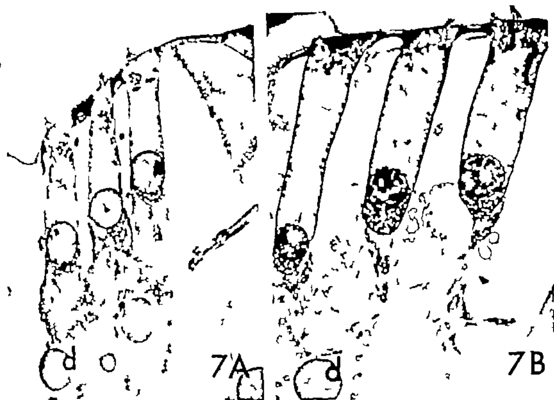


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Fig. 10. Stereocilia of outer hair cells 6 h after KAN/EA. The stereocilia are distorted and the plasma membrane has lifted off their fibrous axial cores. × 10 000.

cisternae and clumping of nuclear chromatin. However, in contrast to the findings of Lundquist & Wersäll (1966) the mitochondria appeared unchanged. Examination of earlier time periods will be necessary to determine whether the membrane or nuclear alterations occur first.

The speed and severity of the ototoxicity produced by the interaction between single doses of kanamycin (400 mg/kg) and ethacrynic acid (40 mg/kg) compared with the 12 or more days required to produce ototoxicity in guinea pigs given daily doses (400 mg/kg) of kanamycin alone (Lundquist & Wersäll 1966) leads to the speculation that the interaction effects are due to an acceleration of kanamycin ototoxicity by ethacrynic acid. One hypothesis we propose is that ethacrynic acid alters the permeability of the hair cells, thereby allowing more kanamycin to enter the cells. When administered to animals by itself kanamycin produces its ototoxic effects only

Fig. 11. Organ of Corti 4 h after KAN/EA. All three rows of outer hair cells in the basal turn have been selectively destroyed (*). The pillar cells (p) have collapsed and the tunnel filled in by adjacent cells. × 400.

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Fig 8 Plasma membrane of outer hair cells 3 h after KAN/EA The sub-surface cisternae which normally lie along the plasma membrane of each outer hair cell have begun to disappear (*arrow*) particularly in areas where the cell has expanded ×2000

drug therapy and have determined that hair cells are damaged within several hours of treatment with ethacrynic acid when preceded by kanamycin At 3 h after KAN/EA the OHCs of the basal turn of the cochlea are already dying and by 24 h most of the OHCs are completely gone

The pattern of the OHC damage and early appearance of nuclear changes was similar to that found by others in studies of the ototoxic effects of large daily doses of kanamycin alone (Kohonen 1965 Lundquist & Wersäll 1966 Ostyn & Tyberghein 1978) and other aminoglycoside antibiotics (Kohonen 1965 Ostyn & Tyberghein 1968 Wersäll et al 1973 Ylikoski et al 1974) Damage had progressed the furthest in the basal regions and least in the more apical region Although we found no damage to the inner hair cells by 24 h after KAN/EA an early study (West et al. 1973) found that most IHCs were gone 30 days after KAN/EA The reason for this delay in IHC death is unknown

The earliest ultrastructural changes we observed occurred in the nucleus and plasma membrane of the outer hair cells of the basal turn As early as 3 h after KAN/EA we observed changes in the plasma membrane accompanied by a loss of the lateral subsurface

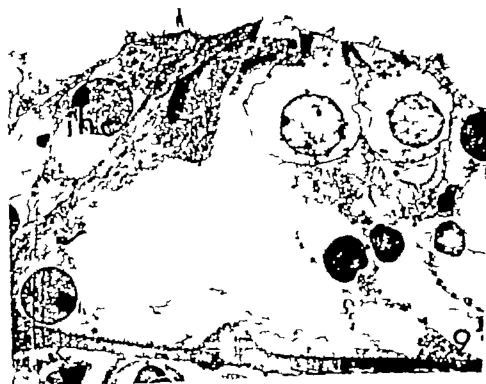


Fig 9 Organ of Corti 6 h after KAN/EA. The outer hair cells show severe damage whereas the inner hair cell (*ihc*) looks normal All membranes of the outer hair cells show significant damage and each cell and its nucleus are grossly swollen ×1800

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Another feasible hypothesis would be that ethacrynic acid acts directly on the hair cells causing them to die We have recently shown that low concentrations of ethacrynic acid are toxic to mammalian cells in culture (50% growth inhibition at 2 3×10⁻⁵ M [Fox & Brum mett 1978]) Thus the interaction effect could be due to some kanamycin induced changes that expose the hair cells to lethal concentrations of ethacrynic acid

Finally we must consider the possibility that ethacrynic acid and kanamycin are acting indirectly through effects at sites in the cochlea other than the hair cells Such indirect effects may alter ionic or metabolic environ ments upon which the cochlear hair cells de pend for maintenance of function Our ultra structural studies and those of others (Quick & Duvall 1970 Bosher et al 1973 Brummett et al 1977) show that a single intravenous dose of ethacrynic acid produces rapid swell ing of the stria vascularis In addition Bosher et al (1973) have found that the structural changes in the stria vascularis are accompanied by major alterations of potassium and sodium concentrations in the endolymph Such changes in ionic composition of the en dolymph may sensitize the hair cells to the toxic effects of kanamycin or may in themselves lead to hair cell damage The reason that the hair cells are unaffected after a single injection of ethacrynic acid alone may be cause of the relatively short duration of ac of the drug. On the other hand we have sh that the strial effects of ethacrynic acid enhanced in the presence of kanamycin may be that kanamycin prolongs the ef of ethacrynic acid on the stria vasculari well as increasing its magnitude resultin sustained ionic changes which ultimately stroy the hair cells

As yet the site(s) and mechanism(s) of ototoxic interaction of kanamycin and e crynic acid have not been elucidated. We know that the interaction produces rapid cell destruction that first appears as char in the nucleus and plasma membranes of OHCs of the basal turn of the cochlea also know that the interaction has a dram effect on the stria vascularis How these fects combine to produce the interaction mains an intriguing problem

ZUSAMMENFASSUNG

Die Wechselwirkungen von Kanamycin (KAN) und acrynsäure (EA) an der cochlearen Feinstruktur w an Meerschweinchen untersucht. Ethacrynsäure (4 kg) wurde 2 Stunden nach Kanamycin (400 mg/kg) ben und Messungen wurden 3 4 6 und 4 Stunden Ethacrynsäureverabreichung unternommen Kontro tersuchungen mit physiologischer Kochsalzlösung (S wurden wie folgt eingeschlossen. KAN/EA KAN/S SAL/EA und SAL/SAL. Nach 3 Stunden wurden V derungen der Zellkerne und Plasmamembranen äußeren Haarzellen des Cortischen Organs gefunden Zellen waren völlig zerstört nach 24 Stunden. Die in Haarzellen waren nicht betroffen Die Stria ascularis Tieren die KAN/EA oder SAL/EA erhalten ha zeigte nach 3 Stunden starke Schwellung die nac Stunden verschwunden war KAN/EA hatte stärkere Wirkung an der Stria als SAL/EA. Diese Befi stimmen mit dem zeitlichen Verlauf der Wirkungen d Pharmaka auf die endocochlearen Wechsel- und Gl strom-Potentiale überein EA hatte keinen Einfluß a KAN Konzentrationen der Perilymphe

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As yet the site(s) and mechanism(s) of the ototoxic interaction of kanamycin and ethacrynic acid have not been elucidated We do know that the interaction produces rapid hair cell destruction that first appears as changes in the nucleus and plasma membranes of the OHCs of the basal turn of the cochlea. We also know that the interaction has a dramatic effect on the stria vascularis How these effects combine to produce the interaction remains an intriguing problem

ZUSAMMENFASSUNG

Die Wechselwirkungen von Kanamycin (KAN) und Ethacrynsäure (EA) an der cochlearen Feinstruktur wurden an Meerschweinchen untersucht. Ethacrynsäure (40 mg/kg) wurde Stunden nach Kanamycin (400 mg/kg) gegeben und Messungen wurden 3 4 6 und 24 Stunden nach Ethacrynsaureverabreichung unternommen. Kontrolluntersuchungen mit physiologischer Kochsalzlosung (SAL) wurden wie folgt eingeschlossen KAN/EA KAN/SAL, SAL/EA und SAL/SAL. Nach 3 Stunden wurden Veränderungen der Zellkerne und Plasmamembranen der außeren Haarzellen des Cortischen Organs gefunden, die Zellen waren vollig zerstört nach 4 Stunden Die inneren Haarzellen waren nicht betroffen. Die Stria vascularis von Tieren die KAN/EA oder SAL/EA erhalten hatten, zeigt nach 3 Stunden starke Schwellung die nach 4 Stunden verschwunden war KAN/EA hatte eine starkere Wirkung an der Stria als SAL/EA Diese Befunde stimmen mit dem zeitlichen Verlauf der Wirkungen dieser Pharmaka auf die endocochlearen Wechsel- und Gleichstrom-Potentiale uberein. EA hatte keinen Einfluß auf die KAN Konzentrationen der Perilymphe

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and serum, and to correlate perilymph concentration with changes in endocochlear potential

MATERIALS AND METHODS

Chinchillas weighing 450–550 g were anesthetized with sodium pentobarbital 30 mg/kg i p Tracheostomy was performed and the animal was promptyly ventilated with a Narco respirator Paralysis of skeletal muscle was obtained with gallamine triethiodide (10 mg/kg i m). The animal's head was securely fixed and a jugular vein cannulated The round window was exposed and a reference electrode placed in the neck muscles. The endocochlear potential (EP) microelectrode was inserted into the endolymphatic space with a stereotaxic manipulator via the round window through the basilar membrane until the scala media was entered. EP was continuously monitored using a high impedance dual electrometer (W P Instruments, F-223) and a Beckman two-channel strip chart recorder FU was injected via the jugular vein cannula.

Pharmacokinetic studies were carried out by collecting serum and perilymph, at various times after an injection of FU (100 mg/kg i v) Perilymph was collected by careful exposure of the round window membrane using a post auricular incision and removal of fluid samples with small capillary micropipettes Care was taken to avoid blood contamination and perilymph samples found by microscopic inspection to have red blood cells were discarded Samples averaged 5 μl and only one animal was used per sample Blood samples were obtained by venipuncture of the jugular vein using syringe and needle They were centrifuged to separate cells from serum taking care to avoid hemolysis.

Concentrations of furosemide in body fluids were determined by high pressure liquid chromatography and ultraviolet detection at 235 nm. The lower limit of detection by this method was ~5 ng of FU Concentrations were extrapolated from standard curves prepared from known concentrations of FU which were linear from 0 050 to 500 μg/ml Sample volumes were 3–10 μl for perilymph and 50 μl for other fluids Samples prepared from untreated animals showed no interfering substances

Animals were studied 5 15 30 and 60 min after injection of FU The mean and standard error (S E M) of 5 to 12 animals was used to compute each point of the decay curve Assuming first-order elimination kinetics the elimination constant (K_{el}) and half life ($t_{\frac{1}{2}}$) were calculated by least squares fit of the mean data for both serum and perilymph. The slope of each line represents K_{el} $t_{\frac{1}{2}}$ is calculated from $t_{\frac{1}{2}} = \ln 2/K_{el}$

Multiple dose studies were carried out by injecting 7 chinchillas with FU (25 mg/kg intraperitoneally) every 12 hours for 5 days Four animals were alive 12 hours after the last dose At this time the single dose experiment was performed as described previously using 100 mg/kg intravenously and body fluids collected 60 min later The results of this chronic study were compared with naive animals receiving furosemide as part of the pharmacokinetic study Statistical significance was determined by using Student s *T* test.

RESULTS

The levels of furosemide in perilymph and serum of the chinchilla after intravenous injection are shown plotted against time in Fig. 1 The respective K_{el} and $t_{\frac{1}{2}}$ values are 0 0206 min and 33 6 min for serum and 0 00526 *min* and 131 min for perilymph ($P < 0\ 01$) Thus, the elimination from perilymph in the time period studied was only one fourth as rapid as elimination from serum

The concentration of furosemide in perilymph was somewhat higher at 15 min (5 2 μg/ml) than at 5 minutes (4.2 μg/ml) and may reflect incomplete equilibration with serum If only the data from animals studied at 15 30 and 60 min are considered the K_{el} for perilymph increases to 0 00913 min⁻ and $t_{\frac{1}{2}}$ = de

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ELIMINATION KINETICS OF FUROSEMIDE IN PERILYMPH AND SERUM OF THE CHINCHILLA*

Neuropharmacologic Correlates

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Summary This study was done to determine the comparative elimination kinetics of furosemide from chinchilla perilymph and serum and to correlate perilymph concentration with changes in endocochlear potential The elimination kinetics of furosemide (FU) were determined in sera and perilymph obtained from chinchillas injected with 100 mg/kg i v of FU Concentrations of FU exhibited a linear decay pattern in serum and perilymph over the initial 60 minutes The rate of decline of furosemide levels in perilymph was about four times slower than the rate of fall in serum Chronic treatment (25 mg/kg i p every 12 hours) did not appear to influence the level of drug at 60 minutes after a dose of FU (100 mg/kg IV) Chinchillas were also studied following doses of FU ranging from 25–200 mg/kg i v to see the effect on endocochlear potential (EP) A positive correlation was found between FU dosage the maximum millivolt reduction of EP and the time to initiation of recovery of EP The perilymph concentration of furosemide when the EP began to recover was 5 μg/ml (1.5×10⁻⁵ M) Knowledge of furosemide kinetics may ultimately be applied to prevent ototoxicity in patients.

Furosemide (4-chloro-*N* (2 furylmethyl)-5-sulfamyl anthranilic acid) is one of the potent loop diuretics—which have been used clinically for the treatment of congestive heart failure in adults (Kim et al 1971) as well as infants (Ross et al 1978) to reduce edema associated with renal failure (Kleinknecht et al 1976 Vereerstraeten et al 1975) and to manage hypertension (Mroczek et al 1975 Kim et al 1971)

Clinical episodes of transient (Vargish et al 1970 Heidland & Wigand 1970 Venkateswaran 1971 Wigand & Heidland 1971 Bourke 1976) and permanent (Lloyd Mostyn & Lord 1971 Brown et al 1974 Quick & Hoppe 1975 Rifkin et al 1978) hearing loss have been reported Most cases have followed high dose intravenous administration, but Rifkin et al have recently reported hearing loss after oral furosemide (Rifkin et al 1978)

Electrophysiologic studies have demonstrated a dose related fall in endolymphatic potential (Kusakari et al 1978) and cochlear N_1 (Brown & McElwee 1972 Brown 1975) after administration of furosemide with bumetanide (Brown 1976) or ethacrynic acid Cochlear microphonics (Goldman et al 1973) and evoked response audiometry (Jung & Rosskopf 1975) have also been used to study the ototoxicity due to furosemide The mechanisms of ototoxicity due to furosemide are unknown

Brusilow found that furosemide 50 mg/kg given to guinea pigs intravenously caused an increase in sodium concentration and a decrease in potassium concentration of endolymph These electrolyte changes were blocked by pretreatment with propranolol (Brusilow 1976)

Nakashima (1978) has recently reported a blockade of furosemide effect on EP using iodinated contrast dye pre treatment

The present study was undertaken to determine the comparative elimination kinetics of furosemide (FU) from chinchilla perilymph

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* A preliminary report of this investigation was presented at the Committee for Research in Otolaryngology American Academy of Otolaryngology and Association for Research in Otolaryngology September 9 1978, Las Vegas, Nevada.

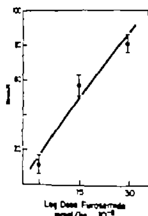


Fig. 3 Maximal mV reduction in EP after various doses of injected furosemide. Each point represents the mean reduction of EP in mV ± standard error

which lasted for a brief period (minutes before full recovery occurred. However doses of 50 and 100 mg/kg reduced the EP to negative values and the EP remained depressed for longer periods of time When the administered dose of FU was plotted against maximal reduction of EP in millivolts a log linear relationship was generated (Fig. 3) The time to initial recovery of EP to positive values can also be expressed as a function of dose Fig. 4)

DISCUSSION

The perilymph levels of FU have been specifically analysed and its pharmacokinetic parameters in this biological compartment computed The prolonged persistence of FU in perilymph when compared with serum may relate to the drug's ototoxicity Slow egress from the perilymph might be explained by trapping of furosemide (a weak acid) in its ionic form, since perilymph is somewhat more alkaline than serum (Ledoux 1943) This would result in a decreased ability of the drug to penetrate membranes bordering on the perilymph. Alternately the long half life may be due to incomplete equilibration of serum and perilymph resulting in continued slow transport from serum into the perilymph

The lack of accumulation in the multiple dose study suggests that most of the drug is eliminated from the perilymph before successive i p doses are given 12 hours later thus resulting in no difference in drug level after the intravenous injection was given

The distinct concentration gradient between serum and perilymph is obvious suggesting a blood–labyrinth barrier for furosemide Since furosemide is 90–95% protein bound in serum (Rane et al 1978) diffusion of only the free fraction into perilymph would be expected to yield concentrations at least 5–10 times those observed

Presumably most of the FU in the perilymph of these experimental animals was derived from blood rather than from CSF since there was such a large concentration gradient favoring diffusion of FU from blood into perilymph On the other hand the concentrations of FU in CSF were never more than one fourth as great as the simultaneous level in perilymph This would make it appear unlikely that FU would passively diffuse from CSF into the perilymphatic space since the concentration gradient favors diffusion of this drug in the opposite direction namely from perilymph to the subarachnoid space Since the FU level is distinctly different in these two fluid com-

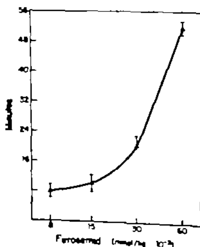


Fig. 4 Time of initial recovery of EP after various doses of injected furosemide. Each point represents the mean time in minutes ± standard error

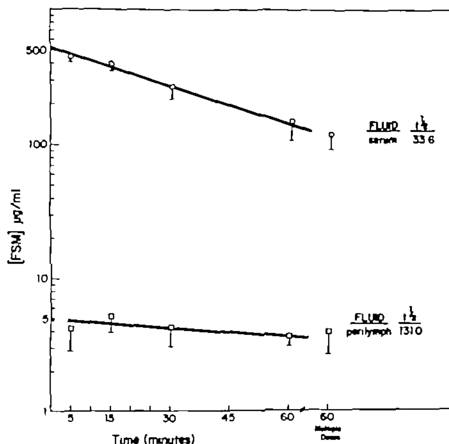


Fig 1 Furosemide kinetics in serum and perilymph after intravenous injection (100 mg/kg).

creased to 75 9 min The difference between this elimination and that in serum however remains significant ($P<0.025$) There was approximately a 100-fold gradient in the FU concentrations of serum and perilymph suggesting the presence of a blood labyrinth barrier for this drug

Concentrations of FU in cerebrospinal fluid (CSF) were also measured in each animal (d[ata] not shown) Cerebrospinal fluid free of blo[od] contamination was obtained by careful pun[c]ture of the atlanto-occipital membrane a[nd] gentle withdrawal using a tuberculin syring[e] FU concentrations in CSF showed a stea[dy] decline from the initial mean peak value [of] 1 4 µg/ml and the rate of decrease was co[m]parable to that of serum ($t_{½}$=22 9 min for CS[F] 33 6 min for serum) Peak concentrations [of] FU in CSF were only about one-fourth [as] great as the peak level in perilymph

Chinchillas treated with FU chronically in [a] dose high enough to produce considerab[le] mortality did not demonstrate any accumul[a]tion of the drug in serum or perilymp[h] [$0.3>P<0.2$ (Fig 1)]

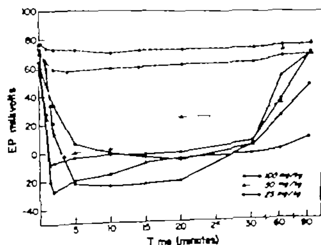


Fig 2 Time course of EP after various doses of injected furosemide

The response of EP as a function of tim[e] following different doses of FU is shown [in] Fig 2 The points at zero time represent th[e] resting EP in the chinchilla which average[d] +65 to +75 mV (somewhat lower than w[as] found in the normal guinea pig) The 25 mg/k[g] dose produced a slight depressant effect on E[P]

der verbrauchten Menge und dem maximalen Potentialabfall (mV) und der Zeitspanne zum Beginn der Erholung des EP. Zum Zeitpunkt der beginnenden Erholung des EP betrug die Konzentration des FU in der Perilymphe 5 µg/ml (1.5 10⁻⁵ M). Es ist erwartet, daß die Kenntnis der Ausscheidungskinetik des FU zur Vermeidung der Ototoxizität bei Patienten ausgewertet werden kann

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partments it does not seem likely that the latter event takes place to any great degree

The lack of accumulation of furosemide after multiple doses may explain the fact that hearing loss associated with this drug is usually temporary in patients and the lack of permanent histologic changes recorded after multiple doses in animals (Federspil & Mausen 1973 Forge 1976) The electrophysiological response of the cochlea in terms of EP after various doses of furosemide administration is demonstrated in this study

The EP behavior over time after various doses of furosemide injection also demonstrated that the EP remained depressed for longer periods of time with higher doses in the chinchilla Kusakari et al (1978) reported similar findings in guinea pigs

A linear relationship between log dose of furosemide and maximal millivolts reduction of EP was observed Similar findings with another loop diuretic (bumetanide) in cats have been reported (Brown 1976) It is interesting to observe that the time required for the initiation of EP recovery is also dose related

The perilymph concentration of furosemide extrapolated from the kinetic study at the time corresponding to EP recovery after the 100 mg/kg dose was approximately 5 μg/ml (1.5×10^{-5} M)

These new findings suggest that the functional disturbances of the cochlea may be closely related to the amount of drug administered There seems to be a threshold blood level which may cause a functional disturbance of hearing The precise threshold in patients is not known

In human studies when a massive dose of FU was given over a short period of time a reversible hearing loss was recorded audiometrically (Heidland & Wigand 1970) On the other hand when FU was given by slow infusion (5 6 mg/min) no audiometric changes could be detected These findings suggest that an abrupt elevation of serum concentration may increase the concentration of FU in perilymph resulting in the measured function disturbances on audiograms

There have been two reports that sugge ototoxic blood levels of FU In the first pape patients achieving blood levels greater th 100 μg/ml were said to be at a risk for perm nent deafness (Brown et al 1974) Howeve some of these patients were also receivi aminoglycoside antibiotics The second repo states that a serum concentration of FU abo 50 μg/ml was associated with a high inciden of auditory disturbances (Beerman et a 1977) No audiometric documentation w provided however Since our study demo strated dose related cochlear disturbance and that furosemide is transported into t perilymph the risks of high blood level affe ing hearing are partially explained Furth audiological studies of patients receiving va ous doses of FU with measurement of blo levels are warranted

The present study has demonstrated do related changes in EP after FU administrati in chinchillas Furthermore the establishme of a quantitative relationship between the fur semide concentration of blood and perilym provides a possible pharmacokinetic means predicting the threshold concentration of fur semide in perilymph by measuring the blo concentration

ZUSAMMENFASSUNG

Die Untersuchung wurde unternommen zur Bestimm der vergleichenden Ausscheidungskinetik des Fu semide (FU) aus der Perilymphe und dem Blutser in Chinchillas und fernerhin um die Perilymphe Ko zentration mit Veranderung im endocochlearen Pot tial (EP) zu korrelieren. Nach der intravenösen Inj tion on FU (100 mg/kg) wurde die Ausscheidungskine des FU in Blutserum und Perilymphe von Chinchil bestimmt. Die Spiegel der FU Werte zeighten linea Abfall in Perilymphe und Blutserum Während der ers 60 Minuten. Die Geschwindigkeit des Abfalls des F Spiegels in der Perilymphe war ungerfähr 4mal langsa als der Abfall im Serum Chronische Verabreichu (25 mg/kg i.p alle 1 Stunden) hatte keinen Einfluß a den Serumspiegel oder Perilymphspiegel des Arzn mittels 60 Minuten nach einer intravenösen Gabe FU (100 mg/kg) Nach verschiedenen intra cods Gaben von FU (25–200 mg/kg) wurde auch das EP geme sen Eine positiv Korrelation wurde gefunden zwisch

Table I. *Percentages of ears of differing tympanogram type at different ages*

Tympanogram type	2–4 days (N=300) %	3 months (N=252) %	6 months (N=238) %	9 months (N=236) %	12 months (N=206) %
A	90	82	62	47	40
C_1	10	17	27	38	28
C_2		1	10	11	19
B			1	4	13

on suspicion of ear disease. From the time when the infant was 3 months of age the parents kept a daily record of the duration and severity of catarrhalia, just as other febrile disorders, diseases and possible antibiotic treatment were registered.

The parents of 32 infants (16 boys and 16 girls) did not wish to participate further in the investigations but the withdrawal occurred primarily (26 children) within the first 6 months. Since furthermore, not all participants appeared at all investigations the material has gradually been somewhat reduced (Table I). However 90 children (40 girls and 50 boys) have regularly been examined.

RESULTS

As in previous studies each ear was considered individually and for practical reasons the middle ear pressures were subdivided into four types, type A middle ear pressure from 0 to −99 mmH₂O, type C_1 −100 to −199, type C_2 −200 to −350 mmH₂O and type B flat curve without an impedance minimum.

The results obtained at birth and at 3 and 6 months after birth have been discussed in detail earlier (Poulsen & Tos 1978) (Table I).

At 9 months the tympanograms were significantly worse. Only 47% of the ears had a type A tympanogram (Table I) which is highly significant (χ^2 test $p<0.001$) compared with the evaluation at 6 months. In contrast, the increase from 1% to 4% of the type B tympanogram is not significant.

At 12 months a significant increase in the type B tympanogram ($p<0.01$) was found compared with the evaluation at 9 months. In addition there was a further reduction—though not significant ($p>0.05$)—in the type A tympanogram, such that only 40% of the ears had normal tympanometry at the age of 1 year (Table I).

Seven children (5 boys and 2 girls) with flat curves at two investigations at least were tubulated on the right ear and had paracentesis performed with evacuation of mucus in the left ear between the 9- and 12-month investigation. In all of these children secretory otitis with mucous secretion and a thickened middle ear mucosa was found in the middle ear. At the 12-month investigation these chil-

Table II. *Percentages of ears of differing tympanogram type in girls and boys at different ages*

Tympanogram type	2–4 days Girls (N=136) %	2–4 days Boys (N=164) %	3 months Girls (N=106) %	3 months Boys (N=146) %	6 months Girls (N=108) %	6 months Boys (N=130) %	9 months Girls (N=102) %	9 months Boys (N=134) %	12 months Girls (N=94) %	12 months Boys (N=112) %
A	92	87	77	86	65	6[illegible]	49	46	39	41
C_1	8	13	1	14	25	27	39	37	4	30
C_2			2		9	10	10	11	28	1
B					1	1	2	6	9	17

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SCREENING TYMPANOMETRY DURING THE FIRST YEAR OF LIFE

M Tos G Poulsen and A B Hancke

(Received December 4 1978)

Abstract 150 healthy children were regularly investigated with tympanometry during the first year of life Normal middle ear pressure was found in nearly all children at birth. At the age of 6 months 62% of the ears had a pressure of 0–99 mmH_2O In 37% the pressure was between −100 and −350 mmH_2O and 1% had flat curves. At the age of 9 months the tympanograms further deteriorated and at 12 months only 40% of the ears had a pressure of 0 to −99 mmH O 28% had a pressure of −100 to −199 mmH_2O 19% a pressure of −200 to −350 mmH O and 13% had flat curves indicating secretory otitis At 1 year the tympanograms were worse than in any other age group investigated so far The dominant cause of the reduced middle ear ventilation was catarrhalia, the frequency of which increased during the period from 6 to 12 months

Relatively few children are treated for secretory otitis at the age of 1 year This may either be because the disease is not yet very frequent and not fully developed or because it does not give symptoms and is not diagnosed In order to determine the frequency of secretory otitis and tubal dysfunction the time of the onset of the disease and its etiology we have performed tympanometry in a group of healthy children at regular intervals from birth

The results of the tympanometric examination in newborns and at follow up investigations 3 and 6 months after birth have been published earlier (Poulsen & Tos 1978) In the present study the results of the investigations at 9 and 12 months are presented and correlated to the tympanometric findings during the first six months of life (Table I) Similar investigations of 1 year-old children have not been published earlier Among healthy 2 year-old children we found flat curves in 10 8% of the ears and in 39 5% a middle ear pressure of −100 to −350 mmH (Tos et al 1978*a*) At the second investig tion 2 months later we found flat curves i 14% of the ears and a middle ear pressure (−100 to −350 mmH_2O in 38 6% Howeve the ears were not the same as in the fir investigation as 52 8% of the ears had a di ferent type of tympanogram (Tos et al 1978*b*) in 26% it had improved and in similar percentage it had deteriorated Th type B tympanogram was improved in 50 of the ears indicating a large spontaneo recovery from secretory otitis Similar re sults were obtained among 3-year-old childre (Fiellau Nikolajsen et al 1977 Fiellau-Nik lajsen & Lous 1979) In 7 year-old childre the tympanograms were significantly bette only 13% of the ears had a pressure belo −100 mmH_2O of these 2% had flat curv (Renvall et al 1975)

MATERIAL AND METHOD

The material originally comprised 151 health newborn infants 82 boys and 69 girls bor consecutively from January to April 1977 a the maternity ward of Gentofte Hospital Some 2–4 days after birth tympanometry wa performed with a Madsen ZO70 impedanc apparatus For practical reasons *the middl* ear pressure was round off to the neares 25 mm interval e g 0 25 50 75 100 mmH_2O etc Tympanometry was systematically re peated every third month and furthermore the parents were requested to appear for a further examination if catarrhalia occurred o

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(Received December 4 1978)

Abstract 150 healthy children were regularly investigated with tympanometry during the first year of life Normal middle ear pressure was found in nearly all children at birth At the age of 6 months 62% of the ears had a pressure of 0–99 mmH$_2$O In 37% the pressure was between −100 and −350 mmH$_2$O and 1% had flat curves At the age of 9 months the tympanograms further deteriorated and at 12 months only 40% of the ears had a pressure of 0 to −99 mmH$_2$O 28% had a pressure of −100 to −199 mmH$_2$O 19% a pressure of −200 to −350 mmH$_2$O and 13% had flat curves indicating secretory otitis At 1 year the tympanograms were worse than in any other age group investigated so far The dominant cause of the reduced middle ear ventilation was catarrhalia, the frequency of which increased during the period from 6 to 12 months

Relatively few children are treated for secretory otitis at the age of 1 year This may either be because the disease is not yet very frequent and not fully developed or because it does not give symptoms and is not diagnosed In order to determine the frequency of secretory otitis and tubal dysfunction the time of the onset of the disease and its etiology we have performed tympanometry in a group of healthy children at regular intervals from birth

The results of the tympanometric examination in newborns and at follow up investigations 3 and 6 months after birth have been published earlier (Poulsen & Tos 1978) In the present study the results of the investigations at 9 and 12 months are presented and correlated to the tympanometric findings during the first six months of life (Table I) Similar investigations of 1 year-old children have not been published earlier Among healthy 2 year-old children we found flat curves in 10 8% of the ears and in 39 5% a middle ear pressure of −100 to −350 mmH (Tos et al 1978*a*) At the second investi tion 2 months later we found flat curves 14% of the ears and a middle ear pressure −100 to −350 mmH$_2$O in 38 6% Howev the ears were not the same as in the fi investigation as 52 8% of the ears had a d ferent type of tympanogram (Tos et a 1978*b*) in 26% it had improved and m similar percentage it had deteriorated T type B tympanogram was improved in 50 of the ears indicating a large spontaneo recovery from secretory otitis Similar r sults were obtained among 3-year-old childr (Fiellau Nikolajsen et al 1977 Fiellau-Niko lajsen & Lous 1979) In 7 year-old childre the tympanograms were significantly bette only 13% of the ears had a pressure belo −100 mmH$_2$O of these 2% had flat curve (Renvall et al 1975)

MATERIAL AND METHOD

The material originally comprised 151 healthy newborn infants 82 boys and 69 girls born consecutively from January to April 1977 on the maternity ward of Gentofte Hospital Some 2–4 days after birth tympanometry was performed with a Madsen ZO 70 impedance apparatus For practical reasons the middle ear pressure was round off to the nearest 25 mm interval e g 0 25 50 75 100 mmH$_2$O etc Tympanometry was systematically repeated every third month and furthermore the parents were requested to appear for a further examination if catarrhalia oc

Table I. *Percentages of ears of differing tympanogram type at different ages*

Tympanogram type	–4 days (N 300) %	3 months ($N=252$) %	6 months ($N=238$) %	9 months ($N=236$) %	12 months ($N=206$) %
A	90	82	62	47	40
C_1	10	17	27	38	28
C_2		1	10	11	19
B			1	4	13

on suspicion of ear disease. From the time when the infant was 3 months of age the parents kept a daily record of the duration and severity of catarrhalia just as other febrile disorders diseases and possible antibiotic treatment were registered.

The parents of 32 infants (16 boys and 16 girls) did not wish to participate further in the investigations but the withdrawal occurred primarily (26 children) within the first 6 months. Since furthermore not all participants appeared at all investigations the material has gradually been somewhat reduced (Table I). However 90 children (40 girls and 50 boys) have regularly been examined.

RESULTS

As in previous studies each ear was considered individually and for practical reasons the middle ear pressures were subdivided into four types, type A: middle ear pressure from 0 to −99 mmH$_2$O type C_1 −100 to −199 type C_2 −200 to 350 mmH$_2$O and type B flat curve without an impedance minimum.

The results obtained at birth and at 3 and 6 months after birth have been discussed in detail earlier (Poulsen & Tos 1978) (Table I).

At 9 months the tympanograms were significantly worse. Only 47% of the ears had a type A tympanogram (Table I) which is highly significant (χ^2 test, $p<0.001$) compared with the evaluation at 6 months. In contrast the increase from 1% to 4% of the type B tympanogram is not significant.

At 12 months a significant increase in the type B tympanogram ($p<0.01$) was found compared with the evaluation at 9 months. In addition there was a further reduction—though not significant ($p>0.05$)—in the type A tympanogram such that only 40% of the ears had normal tympanometry at the age of 1 year (Table I).

Seven children (5 boys and 2 girls) with flat curves at two investigations at least were tubulated on the right ear and had paracentesis performed with evacuation of mucus in the left ear between the 9- and 12 month investigation. In all of these children secretory otitis with mucous secretion and a thickened middle ear mucosa was found in the middle ear. At the 1 -month investigation these chil-

Table II. *Percentages of ears of differing tympanogram type in girls and boys at different ages*

Tympanogram type	2–4 days		3 months		6 months		9 months		12 months	
	Girls (N 136) %	Boys ($N=164$) %	Girls ($N=106$) %	Boys ($N=146$) %	Girls ($N=108$) %	Boys ($N=130$) %	Girls (N 102) %	Boys ($N=134$) %	Girls ($N=94$) %	Boys ($N=112$) %
A	92	87	77	86	65	62	49	46	39	41
C_1	8	13	21	14	25	27	39	37	24	30
C_2			2		9	10	10	11	28	12
B					1	1	2	6	9	17

Table III *Alterations of tympanogram type in 180 ears of 90 children from 6 to 9 months of age*

Types at 9 months of age	Types at 6 months of age A (N=105) %	C (N=55) %	C_2 (N=16) %	B (N=4) %	Total at 9-month evaluation (N=180) %
(a) Pe centages of each type separately					
A	56	35	25	25	46
C	33	49	38	–	38
C_2	10	9	19	–	10
B	1	7	19	75	6
(b) Percentages of all 180 ears					
A	33	11	2	1	
C	19	15	3	–	
C_2	6	3		–	
B	1	2			

dren were included in the group with flat curves—they had a flat curve immediately before treatment and will in all probability also have it at the investigation at 12 months since they had secretory otitis

There were no significant sex differences in the frequency of tubal dysfunction and in the incidence of secretory otitis at the ages of 3 6 and 9 months (Table II) At 12 months there tended to be more type B tympanograms in boys than in girls but the differ ence was not significant ($p>0$ 05)

The middle ear pressure was determined in 90 children (180 ears) at all five investiga tions The total distribution of these tympanograms was the same at the different ages as in the whole material (Tables I III & IV) indicating that the children who were withdrawn from the investigation did not have a special distribution of tympanogram types.

The types of tympanogram changed quite often from investigation to investigation During the first 6 months of life especially from 3 to 6 months a deterioration occurred mainly from type A to C_1 (18%) and C_2 (7%) but also an improvement from C_1 to A (9%) (Poulsen & Tos 1978)

Table III shows the change in tympanogram type from *6 to 9 months*

Change of type A of the 105 ears that had

Table IV *Alterations of tympanogram type in 180 ears of 90 children from 9 to 12 months of age*

Types at 12 months of age	Types at 9 months of age A (N=83) %	C (N=68) %	C_2 (N=18) %	B (N=11) %	Total at 1 -month evaluation (N=180) %
(a) Percentages of each type parat ly					
A	58	31	11	9	40
C	29	31	22	9	28
C_2	10	27	44	–	19
B	2	12	22	82	13
(b) Perce tages of all 180 ears					
A	27	1	1	1	
C	13	1	2	1	
C_2	5	10	4	–	
B	1	4		5	

Table V *Alterations of tympanogram type in 180 ears of 90 children 6–12 months of age*

Types at 12 months of age	Types at 6 months of age A (N = 105) %	C₁ (N = 55) %	C₂ (N = 16) %	B (N = 4) %	Total at 12 months (N = 180) %
(a) Percentages of each type separately					
A	45	42	6	25	40
C_1	31	20	38		28
C_2	17	20	38		19
B	7	18	19	75	13
(b) Percentages of all 180 ears					
A	26	13	1	1	
C_1	18	6	3	–	
C_2	10	6	3		
B	4	6	2	2	

a type A tympanogram at 6 months 56% still had type A at the 9-month evaluation while the rest showed a deterioration to C (33%) and C_2 (10%) in 1% to type B (Table IIIa)

Change of type C 55 ears had a type C tympanogram at the 6-month evaluation (Table IIIa) Of these 49% were unchanged 35% improved to type A the remaining deteriorated to type C_2 and B

Change of type C 25% of type C_2 improved to type A 38% to type C while 19% deteriorated to type B (Table IIIa)

Change of type B Of the 4 ears with a type B tympanogram at the 6-month evaluation 3 remained type B (Table IIIa)

In total 52% of the ears did not change type (Table IIIb) while the tympanogram deteriorated in 33% and improved in 17%

The change of tympanogram type from 9 to 12 months was even larger than earlier (Table IV) in total 48% of the ears did not change type while in 36% it deteriorated and in 16% improved (Table IVb) The changes showed the same pattern as earlier 58% of the type A tympanograms remained unchanged the rest deteriorated, mainly to type C and C_2 (Table IVa) Of type C 31% improved 31% were unchanged the rest deteriorated Of type C_2 44% were unchanged 33% improved to types A and C 22% deteriorated to type B

The majority of the ears having a type B tympanogram at the 9-month evaluation remained unchanged at the 12 month evaluation

The total deterioration in tympanogram types from 6 to 9 months (Table V) was considerable Thus the type of tympanogram deteriorated in 46% of the ears improved in 18% while in 37% it was the same at both evaluations (Table Vb) Deterioration to a flat curve occurred especially in ears that had a type C_2 and C tympanogram at the age of 6 months (Table Va)

Catarrhalia and middle ear pressure

The children s parents kept a daily record of the occurrence and severity of any catarrhalia. During the first 3 months 23% of the children have had catarrhalia and the distribution of tympanograms at the 3-month evaluation was significantly worse in this group (Poulsen & Tos 1978) During the period from 3 to 6 months, only 32% of the children did not have catarrhalia, 49% had a few milder episodes of a total duration of one week per month, i e less than one-fourth of the observation period. 20% had many or severe or prolonged catarrhalia. The tympanometry showed the best conditions in the group without catarrhalia, slightly worse in the group with a few catarrhalia, and still worse in the group with many catarrhalia. The difference

Table VI *Incidence of catarrhalia during the period from 3 to 6 months after birth in 118 children related to tympanogram type at the 6-month evaluation*

Tympanogram type	Incidence of catarrhalia			
	None (N=72) %	Few (N=108) %	Many (N=46) %	Total (N=226) %
A	72	59	48	61
C	22	3	28	28
C_1	6	8	17	9
B		1	7	2

between the group without catarrhalia and the group with many catarrhalia is significant ($p<0.02$) while it is insignificant ($p>0.05$) (Table V) between the group with few catarrhalia and the group with many catarrhalia.

From 6 to 9 months only 3% of the children did not have catarrhalia 40% have had a few and the remainder many catarrhalia (Table VI) There were significantly fewer ears with a type A tympanogram ($p<0.001$) in the group with much catarrhalia compared with the group with little catarrhalia.

Also in the period from 9 to 12 months only 3% of the children had no catarrhalia 46% had a little and the remainder many catarrhalia. The 12 month evaluation again showed a gradual deterioration of the tympanograms with increasing frequency of catarrhalia (Table VII) By testing the number of type A tympanograms there were no significant differences between the group with a few catarrhalia and the group with many catarrhalia ($p>0.05$) while there were significantly more ears with a type B tympanogram ($p<0.001$) in the group with many catarrhalia.

DISCUSSION AND CONCLUSION

Tympanometry performed regularly during the first year of life illustrates the very dynamic course of the tubal dysfunction permitting several conclusions to be drawn

(1) The middle ear pressure gradually de- clines from birth at which time it is norm in nearly all children (Poulsen & Tos, 1978 to the age of 1 year when only 40% of th ears have a normal tympanogram while 13% have a flat curve indicating secretory otiti The deterioration occurs especially during th last 6 months of the first year The tym panograms at the age of 1 year are worse tha at any other age hitherto investigated A typ A tympanogram was found in 50% of the 2 year-old children (Tos et al 1978*a*) in 62% of the 3-year-olds (Fiellau-Nikolajsen et al. 1977) and in 86% of the 7 year-olds (Ren vall et al 1975)

(2) The normal middle ear pressure in nearly all children at birth (Keith 1973 Bennett 1975) and in most children during the first 3 months indicates that the cause of the deterioration of the middle ear ventilation during the first year of life is acquisitive The most frequent cause is catarrhalia, which in this age group increases in frequency and where a significant relationship may be demonstrated between the frequency and severity of the catarrhalia and the reduced ventilation of the middle ear (Tables VI VIII) In viral or bacterial rhinitis and rhinopharyngitis the mucosa is swollen with hypersecretion and reduced function of the ciliae which first of all reduce or abolish the ventilation of rhinopharynx and thereby of the middle ear

Table VII *Incidence of catarrhalia during the period 6–9 months after birth in 118 children related to tympanogram type at the 9-month evaluation*

Tympanogram type	Incidence of catarrhalia			
	None (N=8) %	Few (N=94) %	Many (N=134) %	Total (N=[illegible]36) %
A	100	63	35	48
C		[illegible]5	48	37
C_1		1	10	11
B		1	7	4

Table VIII *Incidence of catarrhalia during the period 6–12 months after birth in 108 children, related to tympanogram type at the 12-month evaluation*

Tympanogram type	Incidence of catarrhalia			
	None (N 8) %	Few (N 100) %	Many (N=118) %	Total (N=216) %
A	75	42	30	38
C_1	25	20	30	26
C_2		23	22	23
B		5	19	13

(Tos & Bonding 1977) Secondly the inflammatory changes propagate from the nose and rhinopharynx to the histologically identical mucosa of the pharyngeal part of the Eustachean tube resulting in an internal tubal occlusion. The inflammatory oedema with vessel dilatation of the tubal mucosa together with the hypersecretion from the mucous glands will cause a transient occlusion of lumen and reduced ventilation. When analysing the etiological factors among the 2 year olds (Tos et al. 1979) we also found a highly significant correlation between catarrhalia and deterioration of the tympanometric conditions. When catarrhalia improved from the first to the second investigation the tubal function likewise improved and vice versa (Tos et al. 1978*b*) Also acute otitis causes deterioration of the tubal function.

(3) The changes in middle ear pressure from investigation to investigation (Tables III–V) are comprehensible when related to the etiological factors Catarrhalia, causing a change from type A to C_1 or C_2 at one investigation, may have improved or ceased before the next investigation with consequent improvement in the middle ear pressure Conversely catarrhalia may recur before a normalization has begun thus deteriorating the pressure The variability in the distribution of the tympanograms at the age of 1 year is thus due to a combination of spontaneous improvement of the preceding etiological factors and of the preceding reduced middle ear ventilation just as it is influenced by new etiological factors The deterioration of the tympanogram occurs primarily from type A to C_1 and C_2 the improvement in reverse order The changes in tympanogram types in both 2 year-olds and 3-year-olds are likewise considerable at the different evaluations (Tos et al 1978*b* Fiellau Nikolajsen & Lous 1978)

(4) The percentage of ears with a flat curve and secretory otitis is very small during the first 6 months which is concordant with the secretory pathogenesis of the disease (Tos 1976) It takes some time before a chronically reduced function of the tube manifesting itself as either a type C_1 or C_2 leads to such metaplastic changes of the middle ear mucosa that the increased production of mucus and exudate cause secretion to accumulate in the middle ear At the ages of 9 and 12 months there is a considerable increase in the number of ears with a flat curve since some of those who earlier had a chronic tubal occlusion manifesting itself as type C_2 and C_1 now have a type B tympanogram Tympanometrically there is thus a gradual change from type C_2 to B i e from chronic tubal dysfunction to manifest secretory otitis This gradual transition may also be demonstrated by quantitative histology in a child with chronically reduced tubal occlusion manifesting itself as a type C_2 we found the same qualitative changes (Tos 1979) as in the incipient stage of secretory otitis (Tos & Bak Pedersen 1976) and in manifest secretory otitis with mucous secretion (Tos & Bak Pedersen, 1973 1975) but quantitatively these changes are considerably less pronounced in chronic tubal occlusion than in secretory otitis

(5) There were no significant sex differences regarding the incidence of secretory otitis at the age of 1 or 2 years Among school children Brooks (1977) found more boys than girls with effusion and Davison (1966) found twice as many boys

ZUSAMMENFASSUNG

150 gesunde Kinder wurden während des ersten Lebensjahres regelmäßig mit Tympanometrie untersucht Bei der Geburt wurde bei beinahe allen Kindern normaler Mittelohrdruck gefunden Im Alter von 6 Monaten hatten 62% der Ohren einen Druck von 0 bis −99 mmH_2O 37% von −100 bis −350 und 1% flache Kurve Im Alter von 9 Monaten wurden die Tympanometrieverhält nisse noch verschlechtert und im Alter von 1 Monaten hatten nur 40% der Ohren einen Druck von 0 bis −99 mmH_2O 28% von −100 bis −199 19% von −200 bis −350 mmH_2O und 13% hatten flache Kurve deutend auf eine sekretorische Otitis Beim Alter von einem Jahr waren die Tympanometrieverhältnisse die schlechtesten von allen bisher untersuchten Jahrgangen Die über wiegende *Ursache* der verschlechterten *Ventilation* des Mittelohren war Catarrhalis, dessen Häufigkeit im Alter von 6 bis 12 Monaten zugenommen hat

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THE ROLE OF THE PARS FLACCIDA IN THE MECHANICS OF THE MIDDLE EAR

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Abstract. The role played by the pars flaccida in the functioning of the middle ear is not altogether clear The aim of our research was to study the movements of pars flaccida in altering the air volume in the middle ear By using [illegible] placed either in the tympanic bulla or in the Eustachian tube in the rat, the middle ear can be insufflated or aspirated with exact volumes of air Pars flaccida reacted promptly to the changes, while pars tensa remained immobile. A large air volume caused perforation of the pars flaccida It seems that pars flaccida function may consist in maintaining a constant middle ear pressure within certain limits, by changing its position

After Gabriello Fallopio (1561) described in some detail the structure of the middle ear and the membrana tympani there was a lag of more than 250 years before the eardrum s structure was again investigated In 1832 an Englishman Henry Jones Shrapnell published his article On the form and structure of the membrana tympani He stated that three-quarters of the eardrum s stroma consisted of a relatively inelastic connective tissue plate attached to the hammer-handle and inserted in the sulcus tympanicus This part he termed the pars tensa and considered that it served the purpose of sound transmission. The remaining quarter of the eardrum situated between the hammer folds and the incisura Rivini, just where the sulcus tympanicus is absent, he termed the pars flaccida as a consequence of this membrane part having a completely different structure from the pars tensa. It was limp and upon blowing/sucking air through the Eustachian tube it could be made to bubble out or in in contrast to the rigid pars tensa. The pars flaccida, often called the membrana Shrapnelli after its discoverer was considered not to have anything to do with sound transmission It ought instead to function as a safety bellows preventing the pars tensa from moving under larger pressure or volume change in the middle ear for instance when sneezing, coughing, and also by low frequency noise

In spite of Shrapnell's brilliant work regarding the pars flaccida, it fell almost into obscurity This is all the more remarkable as most pathological ear conditions involve the pars flaccida in particular

Instead greatest interest was concentrated on the eardrum s sound transmitting part, that is the pars tensa. A few reports did appear concerning the pars flaccida but these were rather contradictory Kessel (1892) stated that the pars flaccida was merely a part of the auditory meatus skin which extended over the incisura Rivini Marx (1935) confirmed this viewpoint as he saw connective tissue filaments from the pars flaccida pass diffusely into the auditory meatus skin s subcutis He stated further that the upper part of the pars flaccida is extremely difficult to visualize by otoscopic examination Oltman (1940) stated that lamina propria was entirely absent in the pars flaccida, which observation Hentzer (1969) completely refuted. Tonndorf & Khanna (1970) supported the suggestion of v Békésy (1941) namely that the pars flaccida only plays a passive role in sound transmission

ZUSAMMENFASSUNG

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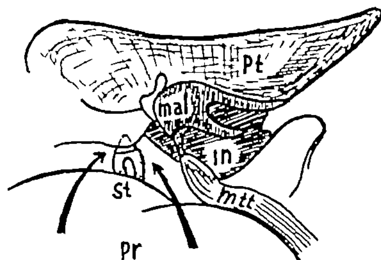


Fig 2 Photograph taken through the operating microscope and schematic drawing showing normal right middle ear of the rat Arrows indicate the passages from the mesotympanum to the atticus (isthmus tympanicus ant et post) Stapes (*st*) musculus tensor tympani (*mtt*) promontorium (*pr*) incus (*in*) malleus (*mal*) pars tensa (*pt*) 10

auditory meatus The pars flaccida is pearl-grey in colour thick and opaque and extremely elastic and allows thus the processus brevis to follow the movements of the pars tensa. The latter by contrast is thin and transparent with a typical fibrous structure (Kirikae 1960)

Anatomically there is great similarity between the rat and human middle ear The tubal opening in the middle ear lies considerably

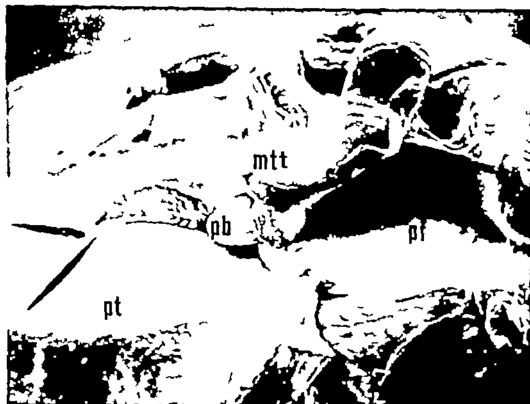


Fig 1 Scanning electron micrograph (SEM) of a normal right tympanic membrane of rat viewed from the middle ear Pars flaccida (*pf*) to the right is bordered by incisura tympanica Rivini processus brevis (*pb*) and is situated lateral to collum mallei (*cm*) Musculus tensor tympani (*mtt*) In pars tensa (*pt*) going from the umbo are seen two fissures caused by the critical-point drying in the preparation procedure for SEM × 4

but that this eardrum part s morphological structure is an absolute necessity to facilitate the required mobility in the pars tensa and the handle

Lim (1968*a b* 1970) was the first to show that there was no elastin to be found in the pars tensa—only collagen while the pars flaccida was rich in elastic filaments He states further that the pars flaccida is thicker and more porous than the pars tensa. Furthermore the pars flaccida does not have so regularly orientated connective tissue architecture

As Cornelius (1825) v Tröltsch (1860) and Proctor (1964 1971) have shown the atticus or epitympanum forms an almost completely separate sac from the rest of the middle ear—a situation brought about by the auditory bone chain and the mucosal folds together with ligaments and muscles The only permanent passages between the atticus and the mesotympanum are as is known the two canals coursing in front of and behind the stapes, viz the isthmus tympanicus anterior and posterior

With the intention of reproducing Shrapnell's observations concerning the structure of the pars flaccida and its physiological function we initiated a study of the pars flaccida, using induced volume changes in the middle ear

MATERIAL AND METHOD

We chose the rat as experimental animal as it has a large pars flaccida which is relatively easy to observe The pars flaccida occupies $\frac{1}{4}$–$\frac{1}{3}$ of the eardrum s area and lies distinctly between the prominent processus brevis on the hammer and the incisura tympanica Rivini which is orientated dorsally in a wedge shape (Fig 1) The pars flaccida continues without any sharp demarcation into the skin of the

RESULTS

With the sond lying either in the bulla tympanica or in the tuba auditiva the position of the enclosed middle ear volume could be altered. An inward displacement of the microliter syringe piston equivalent to a value of 2.5 µl produced maximum bulging of the pars flaccida, while it retracted maximally when 2.5 µl was aspirated (Fig. 4). This effect could be observed in the microscope and documented by photomicrography and cinephotomicrography. The extreme positions could be maintained as long as the air in the middle ear system did not escape. If the animal swallowed however the pars flaccida immediately returned to the normal position. With these positional changes of the pars flaccida, the pars tensa remained virtually immobile.

When the volume increase in the microliter syringe exceeded $+3.5$ µl the pars tensa began to bulge outwards in both the rear quadrants. At the same time as the eardrum could perforate. Such a perforation was always localized to the pars flaccida and was always pointlike, as if a needle had been stuck into an inflated balloon, i.e. it was never ragged or fringed as is the case with a traumatic pars tensa perforation.

DISCUSSION

It can be assumed that temporary pressure variations occur in the middle ear, e.g. when coughing, sneezing, hawking, sniffing, etc. The rat's middle ear is constructed in such a way (see material) that a stream of air via the tuba auditiva passes along the bulla tympanica's rear wall through the isthmus tympanicus anteriorly and posteriorly into the attic. The forepart of the mesotympanum is incompletely demarcated against the rear by the keel-shaped handle and the mass of the tensor tympani muscle. It may be assumed that the pars flaccida functions as a safety valve, a defence mechanism that reacts instantly to pressure variations in the middle ear. A rapid pressure change in the middle ear instantly alters the position of the pars flaccida. Otherwise a pressure increase in the middle ear could be thought to cause lateral displacement of the pars tensa part of the eardrum with the hammer-handle and this could damage the oval window and inner ear. The tensor tympani muscle presumably counteracts such a lateral displacement to some degree. The weakness of the pars flaccida and its way of perforating is reminiscent of the various pathological processes which quite often affect just the pars flaccida and the epitympanum.

In man the pars flaccida is relatively smaller than the corresponding eardrum part in the rat. It would be surprising if the membrana Shrapnelli in man were to completely lack any functional importance and it can be assumed that the human pars flaccida also plays a part in balancing the middle ear pressure.

CONCLUSION

Our studies on the rat's middle ear indicate that the function of pars flaccida is to maintain a constant middle ear pressure within certain limits. An alteration in the middle ear pressure can cause an instant change in the total middle ear air volume through a change in the position of the elastic accommodating pars flaccida.

We hope that further animal studies will make it possible to establish whether pars flaccida's function can have some connection with the origin of retraction pockets and cholesteatoma in the atticus.

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Die Rolle der Pars flaccida in der Funktion des Mittelohrs ist wenig bekannt. Ziel dieser Untersuchung war zu sehen wie sich die Pars flaccida verhält, wenn das Gesamtvolumen des Mittelohrs variiert wurde. Man sonderte entweder die Bulla tympanica oder Tuba auditiva

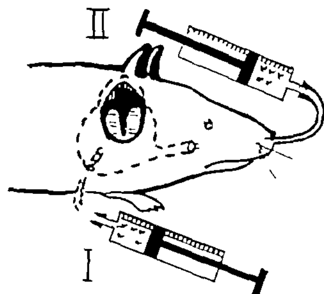


Fig 3 Schematic drawing showing the experimental design Group I (20 rat ears) Air injection from a microlitre syringe through a plastic tube into the bulla tympani Group II (20 rat ears) Air injection into the tuba auditiva Eustachi

more infratympanally in the rat than in man and the entrance to the tympanic cavity is sharply demarcated medially The tensor tympani muscle issues from a bony projection on the neck of the hammer crosses over the promontorium and lower down in front of and medial to the tympanal opening of the tub auditiva. The hammer handle is keel-shape and the frontal part of the mesotympanum thus incompletely cut off by the hammer handle and the belly of the tensor tympar muscle (Fig 2)

The experimental design is shown in Fig 3 Two groups of 20 rats (Sprague Dawley weighing approx 450 g) were studied Intraperitoneal Ketalar® narcosis was used (18 mg/kg bodyweight) In the first group of 20 rats the bulla tympanica was laid bare and the auditory meatus transsected immediately outside the temporal bone thus visualizing the drum The bulla was opened using a drill and a catheter connected to a microlitre syringe was fitted into it

In the second group of 20 rats the auditory meatus was transsected close to the annulus tympanicus In these animals the sond was placed in the tuba auditiva. In order to reach this site the lower jaw had to be resected and the soft palate split The sond in the eustachian tube was connected to a microlitre syringe and the movements of the pars flaccida were visualized through microscope



Fig 4 Photograph of the right tympanic membrane of the rat from the external audit e meatus through the operating microscope ×10 (A) Pars flaccida (*pf*) in normal position. (B) After air insufflation in the middle ear Note the extremely protruded pars flaccida (*pf*) (C) After air aspiration, pars flaccida retract and the middle ear bone tructures can be seen indistinctly behind.

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SYNAPTIC STRUCTURES IN THE TYPE II HAIR CELL IN THE VESTIBULAR SYSTEM OF THE GUINEA PIG

A Free e fracture and TEM Study

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Abstract. The synaptic contacts of the type II hair cell the vestibular system of the guinea pig was described thin-sectioned and freeze-fractured specimens. Synaptic bodies were present at the apposition with both large and small afferent terminals. About 20% of the synaptic bodies observed consisted of complexes of two or more adjacent synaptic discs In freeze-fracture replicas, the cytoplasmic leaflet of the hair cell plasmalemma beneath the synaptic body had bar-shaped aggregate of large particles The size and shape of the particle aggregate was the same as that of the synaptic body Small plasmalemmal deformations, interpreted as sites of synaptic vesicle exocytosis were found immediately adjacent to the particle aggregate. On the postsynaptic membrane, an aggregate of intramembrane particles was present at the synaptic junction The type II hair cell had no gap junctions or close membrane appositions between it and the apposed afferent fiber Efferent boutons ending on the type II hair cell had no intramembrane particle specialization on the postsynaptic membrane however those efferent boutons ending on large and small afferent fibers had an aggregate of medium-sized particles on the external leaflet of the postsynaptic bouton beneath the postsynaptic activ zone

In electron microscopic studies two types of receptor cells are present in the epithelia of the vestibular end organs (Wersäll 1956) A flask-shaped type I hair cell is seen only in higher vertebrates while a cylindrically shaped type II hair cell is found in all vertebrates The two cells are not uniformly distributed in the sensory epithelia. Type II hair cells are more abundant in the periphery than type I hair cells which predominate in the central region of the epithelium. Nerve fibers of different sizes innervate different parts of the sensory epithelium. The central parts of the crista ampullaris and otolith organs receive thick fibers while thin fibers in general terminate in the periphery of the epithelia. As one might predict from these differences in the innervation of the two regions the two hair cells have a different innervation The thick nerve fibers form a calyx around the type I hair cells while the type II cells are contacted by small nerve terminals emanating chiefly from the thin fibers Two patterns of spontaneous activity have been identified in vestibular nerve fibers Fibers from central portions of the sensory epithelia have irregular firing patterns whereas fibers from the peripheral portions of the epithelia have regular firing patterns (Fernández et al. 1971 Goldberg & Fernández, 1972) The irregularly firing units may correspond to the thick fibers innervating the type I hair cell which predominate in the central portion of the epithelium, whereas the regularly firing units may correspond to thin fibers innervating the type II hair cells which predominate in the periphery of the epithelium (Walsh et al 1977) These physiological differences may be related to differences in the synaptic contacts between the two types of hair cells and their afferent fibers (Wersäll 1956 Spoendlin 1966 Hamilton 1968 Wersäll & Bagger Sjöback, 1974 Gulley & Bagger Sjöbäck 1979) The synapse between the type I hair cell and dendritic calyx has very few typical chemical synaptic junctions (Gulley & Bagger Sjöbäck 1979) While gap junctions

der Ratte und konnte eine bestimmte Luftmenge in das Mittelohr einführen oder aus ihm absaugen. Die Pars flaccida reagierte mit einer maximalen Ausbuchtung bzw Einziehung während die Pars tensa sich nicht bewegte Wurde eine größere Luftmenge eingeführt, konnte das Trommelfell reißen und dann in der Pars flaccida. Unsere Auffassung ist, daß die Pars flaccida zur Aufgabe hat innerhalb gewisser Grenzen einen konstanten Druck im Mittelohr aufrechtzuerhalten und zwar durch Änderung ihrer Lage

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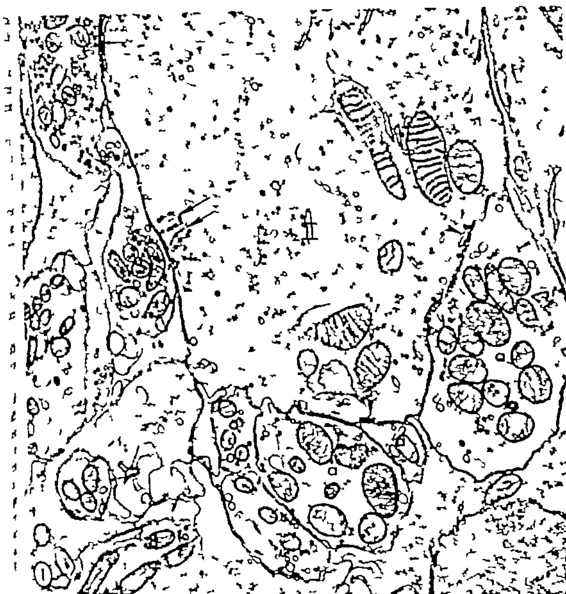


Fig. 1 Thin section through the base of type II hair cell (II) in the crista ampullaris. Five afferent dendrites ( ) and one efferent bouton (*E*) terminate on the hair cell. At the apposition of one of the afferent dendrites, two synaptic bodies (arrow) are present. At the apposition of the efferent bouton and hair cell, cistern of smooth endoplasmic reticulum (*small arrow*) lies under the hair cell plasmalemma. Similar cisterns are seen at other locations along the plasmalemma (*arrow heads*). This bouton is illustrated at higher magnification in Fig. 8. 16720

II hair cells are usually located adjacent to type I hair cells two or more type II hair cells are seldom adjacent to each other in a given section. In contrast to the type I hair cell which is contacted by a single afferent terminal, the type II hair cell is contacted by multiple afferent terminals and also by efferent boutons (Fig. 1) Most synaptic contacts between the type II hair cell and afferent terminals occur at or near the base of the hair cell Occasionally the contour of the cell is very irregular with deep infoldings of the cell membrane

are not present between these two cells other ephaptic interactions are possible at regions of close membrane apposition (Spoendlin 1966 Hamilton 1968 Gulley & Bagger Sjöbäck 1979) In contrast the type II hair cell and its afferent dendrites have only chemical synaptic junctions (Wersäll 1956 Spoendlin 1966 Wersäll and Bagger Sjöbäck 1974)

The current study uses freeze-fractured and thin sectioned material to describe the contacts between the type II hair cell and its afferent dendrites to elucidate further the differences between the synaptic junctions of the type I and type II hair cells

METHODS AND MATERIALS

Fourteen adult NIH strain guinea pigs were sacrificed by intracardiac perfusion with a solution of 0 2 M sodium cacodylate 20 mM calcium chloride and 1% sodium nitrite followed by 3% glutaraldehyde 2% paraformaldehyde 0 1 M sodium cacodylate and 20 mM calcium chloride at 37°C Immediately following perfusion the maculae of the saccule and utricle and the cristae ampullaris of the semicircular canals from both sides were removed and placed in the aldehyde solution at room temperature

The maculae and cristae from 12 animals were prepared for study with the freeze fracture technique After 2 hours in the fixative the tissue from these animals was washed briefly in 0 2 M sodium cacodylate with 20 mM calcium chloride and placed in 20% glycerol in 0 1 M sodium cacodylate for 2 hours The tissue was frozen on golden discs in liquefied monochlorodifluoromethane (Freon 22) and was fractured and replicated at −119°C on a Balzers 301 apparatus with two electron beam guns and a quartz crystal monitor for standardizing the thickness of the replica The replicas cleaned successively in cold methanol Clorox and distilled water were mounted on Formvar and carbon-coated grids and examined in a Siemens Elmiskop 101 electron microscope

Two animals were prepared for thin-secti[on] studies After 12 hours in fixative the tiss[ue] was washed briefly in 0.2 M sodium cacod[yl]ate with 20 mM calcium chloride and po[st]fixed in 1 5% potassium ferrocyanide 1 osmium tetroxide in 0 05 M sodium cacodyla[te] at 4°C The tissue was dehydrated in a grad[ed] series of methanol and embedded in Spurr resin mixture Thin sections were cut a[nd] examined in a Siemens Elmiskop 101 electr[on] microscope

Identification of hair cells and nerve endi[ngs] was accomplished by correlating structu[res] seen in freeze fracture replicas with thin-sec[]tioned material The synaptic contacts of ty[pe] II hair cells were more difficult to identify th[an] similar features in type I hair cells Howev[er] each of the specific features of the hair cell a[nd] dendritic plasmalemma were identified in e[x]amples in which the identification of the c[ell] to which the structure belonged was uneq[ui]vocal

To quantitate the distribution of affer[ent] dendrites on the type II hair cell additio[nal] thin sections from four different cristae amp[ul]lares were cut perpendicular to the apic[al] basal axis of the epithelium and mounted [on] Formvar and carbon-coated slot grids Ea[ch] hair cell in a single section was photograph[ed] at 3000× initial magnification Adjacent se[c]tions were not photographed When sectio[ns] from the same specimen block were used t[he] sections were separated by at least 30 μ[m] Profiles of 72 different type II hair cells we[re] studied The sections used were taken fr[om] both central and peripheral portions of t[he] epithelium No differences were seen in the [in]nervation of the type II hair cells in the d[if]ferent regions of the epithelium

RESULTS

Type II hair cells are principally found in th[e] periphery of cristae ampullares and otoli[th] organs These hair cells are cylindrical a[nd] somewhat longer than the more central[ly] located flasked shaped type I hair cell Ty[pe]

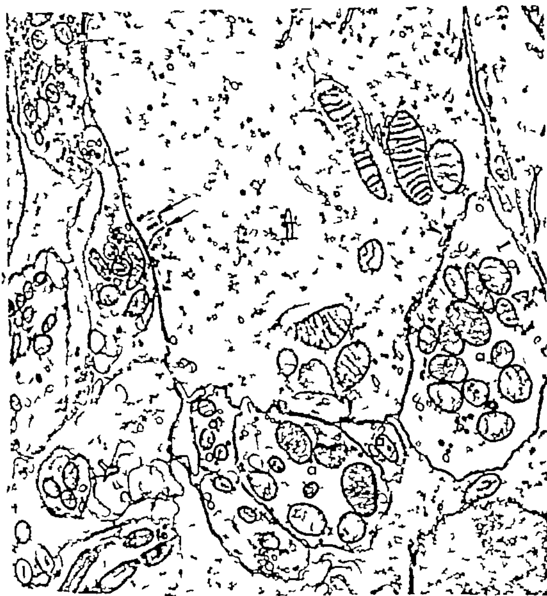


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RESULTS

Type II hair cells are principally found in the periphery of cristae ampullares and otolith organs These hair cells are cylindrical and somewhat longer than the more centrally located flasked shaped type I hair cell Type

Afferent terminals are typically present at the ends of the thick processes formed by the invagination.

Two types of afferent processes contact type II hair cells. A majority of these process es originate from thin afferent fibers and ter minate as small boutons on the hair cell These terminals, containing mitochondria and numerous vesicles of varying sizes are principal ly distributed along the base of the hair cell but are also present near the apex of the cell Large afferent terminals which are collaterals of the thick fibers that give rise to the calyces around the type I hair cell are also present on the type II hair cell. These large terminals envelop part of the base and extend along the side of the hair cell (Fig 2) A single example was seen where the type II hair cell synapsed with the stem of the thick afferent fiber immediately before it formed a calyx with a type I hair cell

Of the 7? profiles of type II hair cells used for quantitation 46 of the profiles had only small afferent terminals The number of small terminals on these cells ranged from one to nine with a mean of 4 0±1 9 boutons/profile The other ?6 profiles of type II hair cells were innervated by a single large afferent terminal and from one to five small afferent terminals (? 6±1 7 small afferent terminals/profile)

The synaptic junction of the hair cell with both types of afferent terminals is the same (cf Figs 1 and ?) The afferent terminal and hair cell are separated by a 25–30 nm extracellular space At the synaptic junction the hair cell is slightly indented by a shallow dome-shaped evagination of the afferent terminal (Figs 3 5) The presynaptic membrane is lined by one or more modified presynaptic densities the synaptic bodies At most junctions only a single synaptic body is present, however at about 20% of both large and small afferent terminals two or more parallel synaptic bodies are present separated by 60–70 nm (Fig 4 A) Infrequently a pair of synaptic bodies is arranged with one synaptic body perpendicular to the other (Fig. 4 C) The synaptic body has a dense fibrillar disc measuring 650 nm in length 60 nm in width and 300 nm in height oriented perpendicularly to the plasmalemma (Fig 3) While this disc is typically solid infrequent examples are found where the disc has a hollow core surrounded by dense fibrillar material (Fig. 4 B) The disc is supported by a base 40 nm in width consisting of two triangular-shaped dense fibrillar pedicles adjacent to the underlying presynaptic membrane The pedicles enmeshed in a fibrillar material are separated by 15–20 nm. The entire synaptic body is surrounded by a single file of round synaptic vesicles measuring about 35 nm in diameter Coated invaginations of the plasmalemma and coated cytoplasmic vesicles are

Fig 3 Thin section through a synaptic body at the apposition of type II hair cell (II) and small afferent terminal (a) At the synaptic junction, the hair cell is slightly invaginated by shallow dome-shaped protrusion of the afferent dendrite and the opposing plasmalemmae are separated by 25–30 nm wide extracellular space The synaptic body consists of pedicle made up of two fibrillar densities (small arrow) embedded in les dense fibrillar network and synaptic disc (arrow) surrounded by synaptic vesicles. Thin fibrillar strands extend between the disc and the surrounding vesicles. A coated vesicle (asterisk) present outside the active zone The post synaptic membrane is lined by prominent postsynaptic density (between arrow heads). 70 400

Fig 4 (A–D) Thin sections through synaptic bodies in the type II hair cell illustrating the variation in the number, morphology and orientation of the synaptic body (A) A complex of three, adjacent parallel synaptic bodies spaced 80-90 nm apart. Each disc is surrounded by shell of synaptic vesicles with adjacent discs sharing file of vesicles The postsynaptic membrane is cut tangentially obscuring the postsynaptic density however postjunctional bodies (arrow heads) are seen (B) Thin section through synaptic disc The plane of section presumably is above and approximately parallel to the junctional membrane The core of the disc is hollow (C) Two synaptic discs, separated by 80–90 nm, are arranged perpendicular to each other A single file of synaptic vesicles at the narrowing region of the discs is equidistant between the two discs (D) A synaptic body is cut approximately perpendicularly along its long axis The synaptic body sits atop a shallow dome-shaped invagination of the hair cell plasmalemma opposite similarly shaped evagination of the afferent terminal. A portion of the pedicle and synaptic disc are present (arrow head) The postsynaptic density (between arrow) extends beyond the length of the synaptic body lining the cytoplasmic aspect of the evaginated dendritic plasmalemma (A) 30 800 (B) 42 40 (C) 44 ?0 (D) 48 400

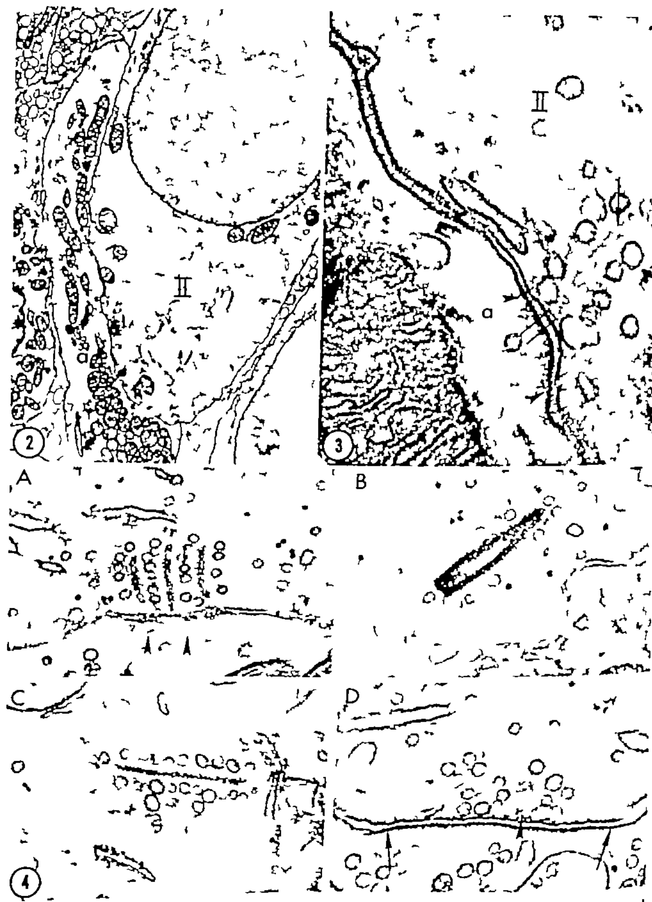


Fig 2 Thin section through type II hair cell (II) i the crista ampullaris A single large afferent terminal (*A*) surrounds the base and side of the cell A comple of two adjacent synaptic bodies is present at a junctional region along the apposition. These synaptic bodies are illustrated at higher magnification in Fig 4 ×4400

common at a distance of about 1–. μm from the synaptic body (Fig. 3)

In freeze-fracture replicas the presynaptic membrane has a band of large intramembrane particles on the cytoplasmic leaflet, directly beneath the synaptic body (Fig. 6) The band measures 600–700 nm in length and 40 nm in width (Fig. 7) and consists of 2 to 4 more or less, distinct rows of particles Small plasmalemma deformations thought to be the site of synaptic vesicle exocytosis during neurotransmitter release (Pfenninger et al 1972 Heuser et al. 1974 Pfenninger & Rovainen, 1974 Gulley 1978) are only found adjacent to the outer row of particles (Figs 6, 7) Large plasmalemmal deformations probably sites of membrane endocytosis in coated vesicles (Heuser et al 1974 Gulley et al 1978) are frequently seen near the band of particles

The postsynaptic membrane beneath the synaptic body is lined by a prominent post synaptic density which extends the entire length of the dome-shaped dendritic evagination (Fig. 4 D) and measures about 150 nm in width (Fig. 3) In freeze-fracture replicas the external leaflet of the postsynaptic membrane beneath the synaptic body has an oval aggregate of large intramembrane particles on the external membrane leaflet. While this aggregate has been identified in many fractures of evaginated afferent membrane apposed to the type II hair cell the critical fracture in which the presynaptic bar of particles or cross fractured synaptic body is visible has not been seen The interpretation of this aggregate as a specialization of the membrane beneath the presynaptic active zone must be tentative

The non-junctional regions of the type II hair cells have a uniform distribution of small- and medium-sized particles on the cytoplasmic leaflet of the plasmalemma. On the external membrane leaflet only scattered intramembrane particles are present In contrast to what is seen on the external leaflet of the type I hair cell (Gulley & Bagger Sjöbäck 1979) no patches of large intramembrane particles are present on the external leaflet of the type II hair cell The plasmalemma of the type II hair cell has no gap junctions or other membrane specialization

Efferent boutons also contact type II hair cells (Figs 1 8) These boutons are small and usually only one ending is found on a hair cell The boutons have numerous round synaptic vesicles approximately 30 nm in diameter These vesicles are tightly packed and clump around small tufts of dense material lining the presynaptic membrane (Fig. 10) The efferent bouton is separated from the hair cell by extracellular space about 25 to 30 nm in width At many efferent to hair cell contacts cisternae of smooth endoplasmic reticulum lie beneath a portion of postsynaptic membrane separated from the membrane by 12 to 15 nm (Fig. 8) The lumen of this cisterna is always patent Similar cisternae are present along regions of the hair cell apposed to afferent dendrites or supporting cells In freeze fracture replicas the active zone of the efferent bouton has numerous large intramembrane particles distributed randomly (Fig 11) Numerous small plasmalemmal deformations are confined to the active zone (Fig. 12) In freeze fracture replicas no specialization of the hair cell membrane is found opposite efferent terminals

Below the hair cells interspersed among the supporting cells efferent boutons also synapse with the afferent dendrites (Fig. 9) These efferent boutons are similar to those on the hair cell (Figs 9 10) The postsynaptic membrane is lined by a thin postsynaptic density (Figs 9 10) however no cisterna of endoplasmic reticulum is present In freeze fracture replicas a loose aggregate of medium-sized particles is present on the external leaflet of the afferent fiber beneath the active zone of the efferent bouton (Fig 13)

DISCUSSION

This study emphasizes those differences between the type I and type II hair cell in addition to the number and size of afferent fibers

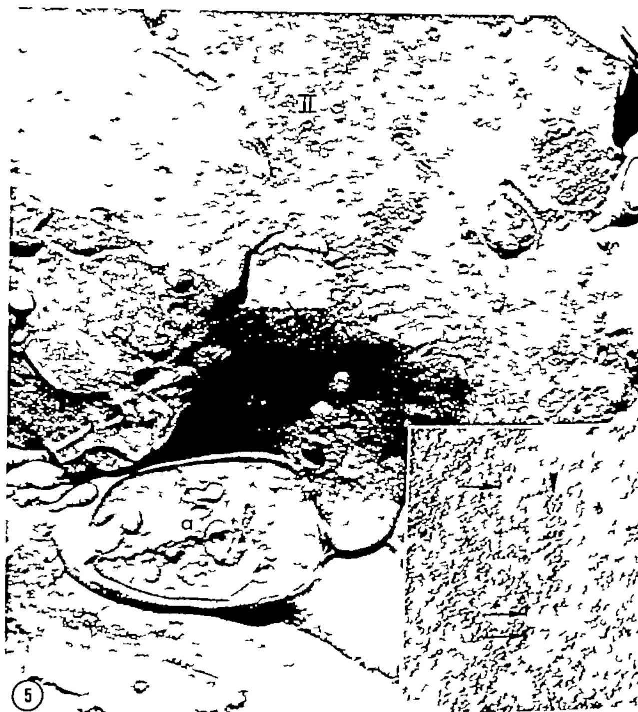


Fig 5 A freeze fracture replica of type II hair cell (II) and an afferent terminal (*a*) The fracture crosses the cytoplasm of the dendrite and exposes small portion of its external leaflet before crossing to the cytoplasmic leaflet of the hair cell and passing across its cytoplasm The fracture across the hair cytoplasm exposes synaptic body (*arrow head*) and associated synaptic esicles adjacent to the fractured membrane leaflet A thin band of large intramembrane particles (*arrow*) lies on the cytoplasmic membrane leaflet immediately beneath the cross-fractured synaptic body Just outside the band of particles two small plasmalemmal deformations (*mall arrows*) are present. Large deformations (*small arrow head* are located away from the active zone A cistern of smoo endoplasmic reticulum (*asterisk*) is apposed to the cyt plasmic leaflet of the hair cell membrane No spec distribution of intramembrane particles is found on th region of the membrane opposed to this cistern *I* se Freeze-fracture replica e posing the cytoplasmic leaflet o a type II hair cell. The fracture exposes a bar of larg intramembrane particles (*arrow head*) at the presynapti acti e zone Three small plasmalemmal deformation (*arrows*) are adjacent to the bar of particles. ×44 500 *Inset* ×84 550.

which innervate them. The type I hair cell is surrounded by a single afferent calyx. Despite the extensive area of this apposition, chemical synaptic junctions between the hair cell and calyx are exceedingly few (Spoendlin, 1966; Hamilton, 1968; Wersäll & Bagger-Sjöbäck, 1974; Gulley & Bagger-Sjöbäck, 1979). In fact, in thin section profiles of 161 different type I hair cells with over 4700 μm of appositional membrane, only six synaptic bodies were observed (Gulley & Bagger-Sjöbäck, 1979). It seems possible that only a single chemical synaptic junction might be present at this extensive apposition. The function of this chemical synaptic junction appears to be augmented or modified by numerous regions where the plasmalemma of the hair cell and calyx are closely apposed. These close appositions are not gap junctions (Hamilton, 1968; Gulley & Bagger-Sjöbäck, 1979); in fact, the plasmalemmata within the close apposition are relatively unspecialized. However, on the external leaflet of the hair plasmalemma, large patches of intramembrane particles surround the regions of close membrane apposition, giving the junction a polarity or asymmetry (Gulley & Bagger-Sjöbäck, 1979). In contrast, the type II hair cell has only chemical synaptic junctions with both small and large afferent terminals. At the apposition of 264 small afferent terminals with about 403 μm of appositional membrane, synaptic junctions consisting of single or multiple synaptic bodies were observed at 110 of the appositions. Of the 110 synaptic bodies, 22 were complexes of two or more synaptic bodies. Of the 26 large afferent terminals with over 88 μm of membrane apposed to the type II hair cell, 18 chemical synaptic junctions were seen. Four of these junctions were complexes of two parallel synaptic bodies. In as much as the large afferent terminals are probably collaterals of afferent fibers that give rise to the calyceal terminal surrounding the type I hair cell, the difference in the number of chemical synaptic junctions per μm of membrane apposed to the different terminals of the thick afferent fiber appears noteworthy. Gap junctions or regions of close membrane apposition are not present between the type II hair cell and its afferent innervation. The external membrane leaflet of the type II hair cell also lacks the patches of large particles present in the membrane of the type I hair cell. While both the type I and type II hair cell utilize chemical synaptic transmission, differences in the den-

Fig. 6. Thin section through an efferent bouton (*E*) and a type II hair cell (II). A cisterna of smooth endoplasmic reticulum is separated from the hair cell plasmalemma by 12–13 nm. The space between the outer cisternal membrane and the plasmalemma does not contain dense material. The lumen of the cisterna is patent. This apposition of efferent bouton and type II hair cell is seen at lower magnification in Fig. 1. × 57 960.

Fig. 7. Thin section through a synapse of an efferent bouton (*E*) on a thin afferent fiber ( ). The synapse is located deep in the epithelium near the point where the dendrite loses its myelin sheath. The postsynaptic dendritic plasmalemma is lined by a thin, postsynaptic density. × 32 300.

Fig. 8. Thin section through an apposition of an efferent bouton (*E*) and an afferent fiber ( ). Small round synaptic vesicles cluster around tufts of dense material (*arrows*) which line the cytoplasmic aspect of the active zone plasmalemma. At the active zone an omega profile (*small arrow*), which is interpreted as a synaptic vesicle which has fused with the plasmalemma, is present. Outside the active zone, a coated plasmalemmal invagination (*arrowhead*) is present. The postsynaptic membrane beneath the active zone is lined by a thin postsynaptic density. × 77 200.

Fig. 9. Thin section through a thick afferent fiber ( ) and two efferent boutons (E_1, E_2). One efferent bouton (E_1) synapses at the branch-point of a small collateral off the thick fiber. The other efferent bouton (E_2) is located on the dendritic stem. × 18 400.

Fig. 10. Freeze-fracture replica exposing the external membrane leaflet of an efferent bouton (*E*) at a synapse with an afferent dendrite ( ). At the active zone, the external leaflet has numerous pits (small arrowheads) corresponding to the large intramembrane particles which presumably remained with the cytoplasmic leaflet during fracturing. Numerous small plasmalemmal deformations (*small arrows*) are confined to the active zone. × 55 200.

Fig. 11. Freeze-fracture replica exposing the external membrane leaflet of a thin afferent dendrite ( ) at a synapse with an efferent bouton (*E*). An aggregate of densely-packed, small intramembrane particles (*arrowheads*) are found on the external leaflet of the postsynaptic plasmalemma. × 41 400.


II
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[...]tiert werden. Kleine Zellmembranimpressionen scheinen an Stelle der ausgedrückten, exocytosen Zellmembranblasen angesehen. Sie kommen im Anschluß an das Partikelaggregat vor. In der postsynaptischen Membrane gibt es ein intramembranöses Partikelaggregat im Gebiet des Synapses. Keine „gap junctions" wurden zwischen Haarzellen Type II und den afferenten Nervenfasern beobachtet. Die efferenten Nervendschlüsse, die in Berührung mit den Haarzellen stehen, haben keine Sonderpartikel in der postsynaptischen Membrane. Die efferenten Nervenendschlüsse, die die größeren oder kleinen afferenten Fasern verbinden, zeigen eine Anhäufung von mittelgroßen Partikeln auf der äußeren Membranhälfte in der postsynaptischen Membran, entsprechend der aktiven Zone der präsynaptischen Membrane.

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sity and number of chemical junctions and the presence of close appositions between the type I hair cell and its afferent calyx may diversify the type of information conveyed by the two receptor cells Data concerning activity at the afferent synapses of the two types of hair cells are lacking (see Goldberg & Fernández 1975) As this problem is approached the differences emphasized in the current study should be useful for interpreting the physiological data

Differences are also present between the synapses of the efferent boutons in vestibular epithelium The efferent boutons in the vestibular epithelium contact type II hair cells and afferent dendrites In thin sections and freeze fracture replicas the presynaptic boutons of both axosomatic and axodendritic synapses are similar however the postsynaptic membranes are different Opposite the axosomatic bouton a cisterna of smooth endoplasmic reticulum does line a portion of the apposition The postsynaptic membrane opposite axodendritic efferent boutons is not lined by smooth endoplasmic reticulum instead a thin postsynaptic density lines the cytoplasmic aspect of the plasmalemma In freeze fracture replicas an aggregate of particles is present on the external leaflet of the plasmalemma opposite the efferent axodendritic bouton A similar specialization is present where the efferent bouton contacts thick afferent fibers or the calyx on type I hair cells (Gulley & Bagger Sjöbäck 1979) The efferent boutons thus appear to have two distinct arrangements of intramembrane particles at the postsynaptic active zone

The postsynaptic membranes of axosomatic and axodendritic efferent boutons are also different in the organ of Corti (Gulley & Reese 1977) There the efferent to afferent dendritic contact has no specialization on the postsynaptic membrane but the efferent to outer hair cell contact has an aggregate of particles on the cytoplasmic membrane leaflet opposite the efferent bouton It has been suggested in the organ of Corti that the two arrangements of intramembrane particles represent different types of ionic channels for mediatating the inhibitory response of the olivocochlear bundle (Gulley & Reese 1977) And indeed following stimulation of the olivocochlear bundle two different physiological responses have been reported—each apparently mediated by a different ionic mechanism (Desmedt & Robertson 1976)

It is also noteworthy that axodendritic contacts in the vestibular epithelium occur in two locations on afferent fibers One occurs on the fiber at the level of the afferent to-hair cell contact on both type I and type II hair cells. The other is found on the stem of both thick and thin afferent fibers central to the point of their branching in the epithelium Thus efferent fibers influence activity in the vestibular epithelium at three levels (1) on the type II hair cell body (2) on afferent terminals near their synapse with the hair cells and (3) on the stem of the afferent fibers near the origin of their myelin sheath Thus as with the case of the synaptic contacts of the type I and type II hair cells the pattern of efferent synapses is complex Underlying this complexity is a morphological diversity which may permit different types of functional interactions.

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Die synaptischen Berührungen an den Haarzellen des Vestibulum der Meerschweine werden an Hand von dünn geschnittenen und gefriergeätzten Präparaten beschrieben Synaptische Körperchen gibt es bei den Synapsen bei den großen und kleinen afferenten Nerventerminalen Fast 70 % der synaptischen Körperchen bestehen aus Komplexen von zwei oder mehreren synaptischen Scheiben. In den gefriergeätzten Repliken gibt es in der inneren Membranhälfte ein bandförmiges Aggregat im Anschluß an die synaptischen Körperchen das aus großen Partikeln besteht Das Format und die Größe des Aggregates kann sehr wohl mit der in den synaptischen Körperchen

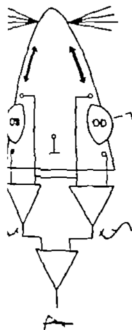


Diagram of the position of the electrodes and recording circuit. The double arrows on the snout ... the respiration movements giving rise to the ... potentials at the first stage amplifier outputs. ... polarity these potentials are made non-... while the potentials resulting from ... (broken lines and arrows) are summated ... eye, OS left eye.)

a clamp in the neck. The body was immobilized by a curved perspex cover which secured with a locking screw the legs freely movable through slots in the table ... this device the alert rat could be tested several hours without any problem.

The electrode position and the preamplifier ... are shown in diagrammatic form in ... 2. Electrodes (stainless steel hypodermic needles) were situated near the outer and inner canthi of the eyes the latter electrodes being isoelectrical with a ground electrode fixed on top of the head

Sinusoidal sometimes even nystagmus-like potentials were observed in the monocular leads originating from snout movements during respiration. By alternating the polarity in both first stage amplifiers and subsequently summating the signals by a third amplifier these respiration artifacts were compensated and the conjugate eye movements were selected. A Tönnies nystagmograph amplifier (*AC-coupled with 2.5 sec time constant*) was used with an Elema Mingograph 81 recorder The respiration movements seen in the monocular leads were continuously monitored on a storage scope and used for controlling the condition of the animal When excessive movements were noted the test was discontinued until the animal had calmed down

As a validity check on the recording technique some experiments were performed on animals in which one or both labyrinths were partially or totally destroyed For destruction of the horizontal canal the periotic capsule was partially exposed in the retro-auricular region and the horizontal canal was transected by drilling a hole in the middle of the inferior margin of the periotic capsule For total labyrinthectomy the middle ear was reached by a retro-auricular approach Thereafter the vestibulum was exposed and its content removed by suction.

Stimulation was performed in complete darkness Between the experiments short pauses were inserted with the lights on in

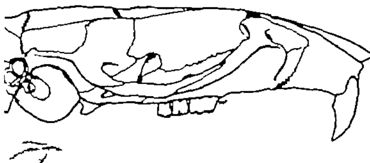


Fig. 3 Drawing of the rat skull showing the position of the semicircular canals and the cochlea.

Acta Otolaryngol 88 412–419 1979

ELECTRONYSTAGMOGRAPHY IN THE LABORATORY RAT

A J E M Fischer P L M Huygen and W Kuppers

From the E.N T Department St Radboud Hospital University of Nijmegen The Netherlands

(Received January 24 1979)

Abstract A method is described for obtaining electronystagmograms from the awake laboratory rat. Threshold values for rotation impulse and oscillatory acceleration were determined as well as the time constant for the horizontal semicircular canal The time constant appeared to be small This might be attributed to the rapid natural head movements in this species No visual suppression of vestibular nystagmus was found in the rat.

The few studies made on the vestibular function of the rat have been performed by visual observation (Griffith 1920 Nylén 1934 T'Ang & Wu 1936 Llinás & Walton 1977) or with neurophysiological methods (Kubo et al 1977 Curthoys 1978) In the present study a method has been developed by which to study vestibular function with electronystagmography in awake rats in order to obtain time constant and threshold stimulus values by means of cupulometry and sinusoidal acceleration in the plane of the semicircular horizontal canal

MATERIAL AND METHODS

Adult Wistar rats (180–200 grams bodyweight) were used for this study The anatomical position of the horizontal semicircular canal in relation to external anatomical landmarks was established by means of dissection technique and by filling the canals with Indian ink and subsequently cleaning the skull

An adjustable frame was developed exerting a minimum of stress on the animal and suitable for rats of various sizes (Fig 1) The animal was placed prone on the table the head was fixed with an adjustable ring around the snout

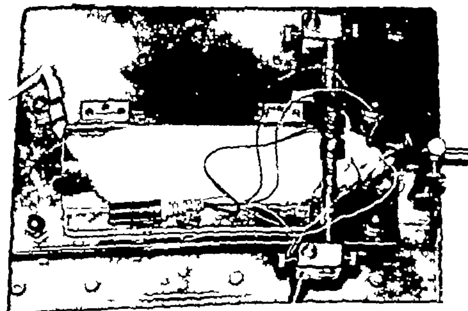


Fig 1 Frame with animal and electrodes in position

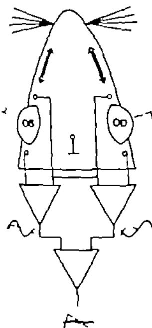


Fig 2 Diagram of the position of the electrodes and the measuring circuit. The double arrow on the snout indicate the respiration movements giving rise to the sinusoidal potentials in the first stage amplifier outputs. By alternating polarity these potentials are made non-coincidental while the potentials resulting from nystagmus (broken lines and arrow) are summated (OD right eye OS left eye)

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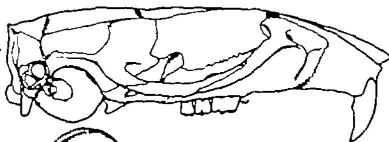


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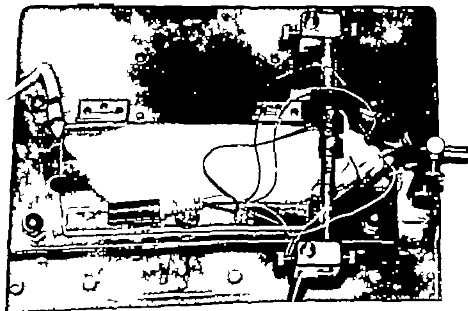
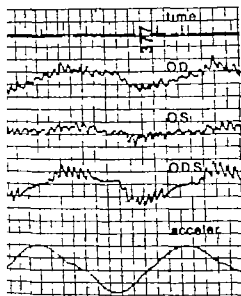


Fig 1 Frame with animal and lectrodes in position



6 Nystagmus provoked by oscillatory stimulation In part of the record, constant amplitude has been led and the maximal angular acceleration is approximately 100 deg/sec²

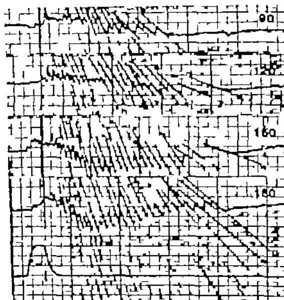


Fig 7 Collage of postrotatory nystagmus after rotation to the left with various velocities (90, 120, 150 and 180 deg/sec from top to bottom). Paper speed 1 cm/sec. The duration of the reaction is measured from the starting point of exponential decline of deceleration (vertical line) to the start of the last measurable slow phase. For measuring nystagmus velocity slope tangents were used in order to calculate decremental time constants as an alternative for cupulometry

stagmus beats versus maximum acceleration per half-period. This method has been described by Montandon et al. (1971) who have shown that the linear regression model is valid in humans.

RESULTS

The anatomical position of the semicircular canals in the skull is shown in Fig 3 The angle between the horizontal canal and the vertical canals appeared to be not significantly different from 90 degrees The mean angle between both vertical canals was 96 degrees (S.E.M 1.5 $n=5$) The mean angle of inclination of the horizontal canal with a horizontal plane supporting the tympanic bullae and the molar teeth was 31 degrees ($n=6$) The plane of the horizontal semicircular canal was not perpendicular to the midsagittal plane A lateral (upward) tilt of the horizontal canal (approx 10 degrees $n=6$) was observed

Fig. 4 shows the nystagmoid respiration movements derived from the separate leads and after summation by the coincidence circuit. These artifacts are almost entirely eliminated after summation enabling the evaluation of nystagmus elicited by vestibular stimulation. This is shown in Figs 5 and 6 An example of the stimulus–response relationship of the postrotatory nystagmus is shown in Fig. 7 A second postrotatory phase was never observed.

From the nystagmus records cupulograms were derived (Fig. 8) In Table I the mean threshold and time constant calculated from cupulograms in all 10 animals are presented No significant correlation between threshold and time constant estimates was observed. The latter appeared to be in good agreement with the values obtained from the decay of slow phase velocity in individual postrotatory reactions the velocity values being obtained from graphical estimates of slope (Fig. 7)

No difference in reaction was observed

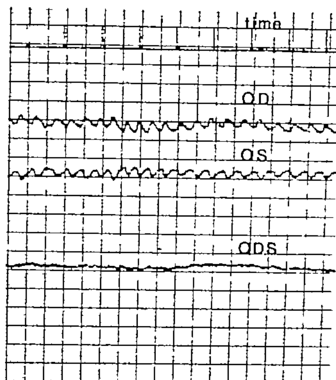


Fig 4 Nystagmus-like potentials caused by respiration movements as shown in the monocular leads (OD OS) These non-conjugate movements are eliminated from the coincidence trace (ODS) *Top trace* time scale in seconds.

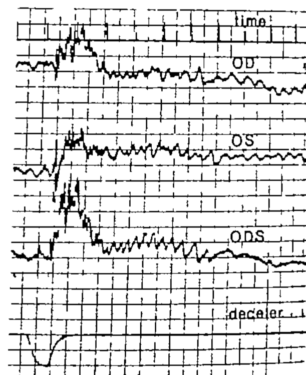


Fig 5 Postrotatory reaction after sudden arrest from 180 deg/sec constant angular rotation Bottom trace deceleration while braking the chair s rotation (200 deg/sec²) The first few seconds only an alarm response is seen then followed by a nystagmus which is only clear from the coincidence trace (ODS) Rotation to the right, nystagmus to the left.

order to stabilize the corneoretinal potential For vestibular stimulation a Tönnies apparatus was used For cupulometry tests constant velocity rotation (40 60 90 120 150 or 180 deg/sec) was obtained by applying a subthreshold acceleration of 4 deg/sec² Sinusoidal oscillation with a fixed period of approximately 6 3 (2π) seconds and growing amplitude was obtained by setting the rotation equipment in the constant velocity pendular mode with an acceleration of 1 deg/sec² Maximum accelerometer readings were recorded for each half period to enable the determination of the threshold acceleration Two growing amplitude tests were performed to see whether response decline occurred In another experiment some animals were submitted to 10 consecutive growing amplitude tests in each of which maximum acceleration was maintained for 1 minute Between the tests pauses of 2 minutes were inserted with alerting conditions and the lights on Oscillatory stimulation was performed in total darkness and in full light alternately in order to see whether visual suppression of vestibular nystagmus occurred. This was also investigated by switching on and off an otoscope light mounted on the revolving chair in front of the animal Moreover it was attempted to elicit optokinetic nystagmus by placing the animal in a revolving optokinetic drum (diameter 50 cm) or by moving a small otoscope light on a distance of 25 cm from the animal's head

The threshold impulse and the long time-constant were obtained from the cupulograms (van Egmond et al 1948) by regression analysis In some experiments the time constant was also measured from the exponential decay of the postrotatory slow phase velocity (Groen 1956–57) A least squares estimate of the sinusoidal acceleration threshold was obtained from a semilog plot of the number of

Table I *Threshold intensity and decremental time constant for the horizontal semicircular canal*

	Threshold (deg/sec)	Time constant (sec)
Cupulometry (10 animals)		
Mean	30 6	3 7
Standard deviation	12 0	1 6
Oscillation (6 animals)	Threshold (deg/sec²)	
Mean	22 5	
Standard deviation	14 4	

the laboratory rat. No signs of distress were observed, even during prolonged sessions (more than 3 hours) Confirmation of the validity of the recording technique could be derived from the experiments on the animals with defective labyrinths By applying controlled vestibular stimulation for which the usual clinical rotation equipment is suitable and using well-founded analytical methods such as cupulometry which has almost fallen into disuse for clinical purposes biophysical characteristics of the semicircular canals can easily be obtained

From the anatomical observations it appeared that the horizontal canal in the white rat inclines at a mean angle of 31 (range 26–37°) to the horizontal plane supporting the tympanic bullae and the molar teeth This agrees with the observations made by Cummins (1924) who reported a mean angle of inclination of 28 4° (range 23–35° in 6 canals) with the plane of the basioccipital bone the latter being approximately coplanar with the horizontal plane defined before However in contrast to the observations made by Cummins (1924) in our study a lateral (upward) tilt of the horizontal canal of about 10° was observed This inclination was of no consequence for the determination of the threshold because the effective stimulus for the pair of the horizontal canals is not less than 0.98 of the stimulus applied (cosine 10)

In the repeated oscillation experiment no response decline was observed This agrees with the observations in humans made by Greiner et al (1970) that response decline never occurs in normal subjects when pendular stimulation is applied In limited amplitude oscillation the absence of response decline has been reported for the cat (Cramer et al 1963) In the rabbit, similar findings have been reported with both prolonged low amplitude stimulation (Kleinschmidt & Collewijn 1975) and repeated high amplitude stimulation (Moser 1978)

A remarkable finding was the lack of any difference in reaction as regards the various illumination conditions This agrees with the observations made by Evlatar & Goodhill (1968) in rabbits Mowrer (1935) however observed a reduction of the duration of postrotatory head nystagmus in pigeons in full light in comparison with complete darkness Fixation suppression as such has been studied in primates (Takemori 1975) The effectiveness of visual suppression might be questioned in afoveate animals which do not have a genuine optical fixation In addition it must be mentioned that according to Griffith (1920) "the lack of distant vision and the probable absence of all clear-cut retinal images (in the white rat) seem to provide the optimal conditions of non-fixation" This might offer another explanation for the lack of consistent optokinetic nystagmus

An old controversy exists with respect to the dimensions in which threshold values for the canal system should be expressed Threshold acceleration values are often reported although it has been repeatedly emphasized that the latency period of reaction is pertinent. Reproducible threshold values can be obtained by applying the Mulder product being the product of latency period and acceleration, because of the integrating properties of the cupula–endolymph system. This system is believed to act as a velocity transducer in the frequency range of natural head movements (van Egmond et al. 1949· Groen, 1956–

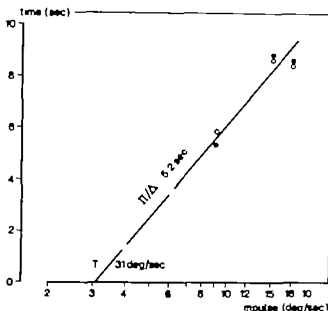


Fig 8 Example of a cupulogram partly obtained from the postrotatory reactions shown in Fig 7 The threshold impulse (*Tr*) is derived from the intercept on the logarithmic abscissa (zero duration of reaction) The time constant is presented as the ratio of the viscous drag coefficient of the cupula–endolymph system (Π) and (Δ) the torque coefficient on this system about the centre of the semicircular canal duct, both derived from Steinhausen s mechanical torsion pendulum model (○ right ● left beating nystagmus)

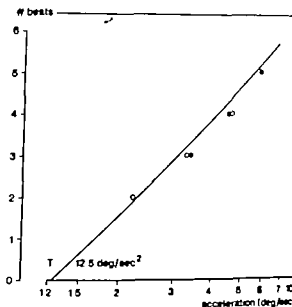


Fig 9 Example of a plot of the number of nystagm beats per half-period against angular acceleration. T threshold (*Tr*) is derived from the intercept on the a scissa. (○ right, ● left beat.)

when the animal was stimulated in complete darkness in full light surrounded by an illuminated optokinetic drum or with an otoscope light in front of the head these devices being mounted on the revolving chair It could be visually confirmed that the nystagmus was purely horizontal and that the reaction intensity declined very rapidly even after the strongest rotation impulse With the techniques used no clear-cut optokinetic nystagmus could be evoked

Growing amplitude oscillation was performed in 6 animals An example of the stabilized response is shown in Fig 6 Ascending threshold estimates were obtained as shown in Fig 9 The mean threshold value is presented in Table I In none of the (5) animals submitted to repeated oscillation was a response decline observed Apart from the nystagmus beats compensatory eye movements occurred on oscillatory stimulation (Fig 6) The latter type of eye movement was also observed w below the nystagmus threshold

During the first week after unilateral de struction of the horizontal canal a spontaneou nystagmus towards the intact side was foun in the prone position and a concordant dire tion-fixed positional nystagmus in the later positions The nystagmus was not visuall suppressed enabling the direct observation o a purely horizontal nystagmus conjugate i both eyes Rotational stimulation revealed nearly complete absence of nystagmus to wards the defective side while incidentall a strong suppression was found Stimulatio of the animals which underwent bilateral laby rinthectomy failed to evoke any nystagmus like reaction Only the usual nystagmoid res piration movements were derived from th separate leads

DISCUSSION

The present experiments clearly demonstrat that with a suitable clamping device and a coincidence circuit reliable nystagmograms can be obtained from such a small animal as

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57) From this theory it can be deduced that in the case of sinusoidal stimulation the threshold is equivalent to the threshold obtained by cupulometry and should be specified in velocity dimensions In the present study (stimulus period 2π) the threshold velocity is obtained by the change of dimension deg/sec² into deg/sec in Table I The threshold of oscillation (22.5±14 4 in 6 animals) did not differ significantly from the threshold obtained by cupulometry in the same animals (30 7±15 6)

The observation of compensatory eye movements occurring even at stimulus intensities below nystagmus threshold has also been reported by Moser (1978) According to Baarsma & Collewijn (1974) using a very sensitive method with the Robinson scleral induction coil no genuine threshold appeared to exist for these compensatory eye movements Therefore it must be kept in mind that vestibulo-ocular reflexes are apparently at work under the nystagmus threshold though this largely escapes our attention when using electronystagmography

The short duration of postrotatory nystagmus in the present study (6 5 sec after 180 deg/sec rotation) is consistent with the observations reported by Griffith (1920) (5 6 sec after 240 deg/sec) The value of the long time constant obtained in the awake rat in the present study (3 7±1 6) appeared to be not significantly different from the value found by Curthoys (1978) in adult anaesthetized rats by single-cell recording from horizontal canal primary neurons It is a rather low value in comparison with the time constants obtained in other species (van Egmond et al 1949 Fernandez & Goldberg 1971 Honrubia et al 1971 Baarsma & Collewijn 1974) but it fits in the assumption made by Jones & Spells (1963) regarding the matching of the semicircular canals to the dynamic requirements of various species It may be inferred that small mammals with relatively quick natural head movements must have at their disposal a highly damped semicircular canal system The impulse threshold (30 6±12.0) however seems fairly high compared with the values reported in other species where values between 2 and 11 deg/sec have been reported (van Egmond et al 1949 Groen 1965 Caston & Gribenski 1966)

One can only speculate about the reason for this apparently low sensitivity of the Wistar rat A possible explanation might be the albinism which for example in the cat has been shown to be associated with vestibular malfunction (Waldorf et al 1977) but inconsistent reactions as reported by these authors were not observed in the present study

To sum up it can be concluded that with this experimental set up reliable measurements of vestibular function can be performed in the rat Because the rat is an outstanding standardized laboratory animal with many data available on its metabolism and physiology this animal may give the opportunity for testing the efficacy of antivertigo drugs and assessing toxic or teratogenic effects of drugs on labyrinthine function

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ZUSAMMENFASSUNG

Eine Methodik wird beschrieben womit Elektronystagmogramme bei wachen Laboratoriumsratten dargestellt werden können. Die Reizschwelle des postrotatorischen Nystagmus, die Schwelle des perrotatorischen Nystagmus bei der Pendelprüfung ebenso wie die Zeitkonstante des horizontalen Bogenganges wurden berechnet. Die Zeitkonstante zeigte einen niedrigen Wert. Wahrscheinlich steht dies in Beziehung zu den raschen natürlichen Kopfbewegungen dieses Tieres Eine visuelle Hemmung des vestibulären Nystagmus konnte nicht nachgewiesen werden.

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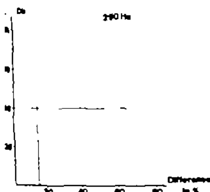


Fig. 1. In Figs. 1-3 the values of unilateral weakness side difference in % (abscissa) are plotted against the hearing loss in dB HL (ordinate). The vertical dotted line indicates the normal limit in caloric reaction (15%), the horizontal dotted line (at 40 dB) divides the hearing loss into lower and greater range. Ordinate: Hearing loss at 250 Hz.

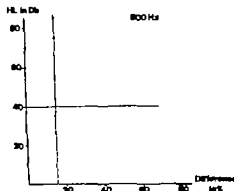


Fig. 2. Hearing loss at 500 Hz (ordinate) against unilateral weakness (abscissa).

4000 Hz. The respective hearing losses for the frequencies of 250, 500, 1000, 2000 and 4000 Hz (in dB HL) were then compared with the results of calorization. We did not average the hearing loss of several frequencies, as the partial information gained by pure tone audiogram would then have been negated. If a contralateral narrow band masking was necessary, then extra care was taken to use neither too little nor too much masking.

(*b*) The well known Fowler test (Fowler, 1936) was used to determine loudness recruitment. The following frequencies were tested: 250, 500, 1000, 2000 and 4000 Hz.

(*c*) The tone decay test (Carhart, 1957) was the third audiological test we performed using the same frequencies as in the Fowler test.

ENG-recorded bithermal caloric tests of both labyrinths

The patients were tested in a darkened room with eyes closed and with their heads fixed in a 60° retroflex attitude. Both labyrinths were then alternately stimulated thermally for a period of 30 sec with water at 30°C and 44°C. The sequence of the stimulations was so chosen that a reverse caloric nystagmus occurred each time. The horizontal nystagmus was recorded with the usual ENG method. The registration of a unilateral weakness or a directional preponderance was obtained by quantitatively calculating the nystagmus as total amplitude in relation to the duration of the nystagmus. This parameter is generally used, and easily recordable, and represents the clinical approximation of the physiological slow component speed parameter. According to many authors the standard deviations of a unilateral weakness obtained with this method fall between 5.3% and 11.5% in the normal population (for the relevant literature see Coats, 1975). The norm limit for unilateral weakness in our clinic is 15%. This means that a test difference of more than 15%

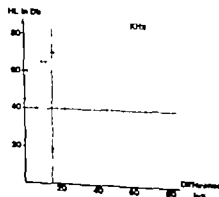


Fig. 3. Hearing loss at 1 kHz (ordinate) against unilateral weakness (abscissa).

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COCHLEO VESTIBULAR CORRELATIONS IN MENIERE S DISEASE

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Abstract The cochlear and vestibular functions were investigated in a sample of 36 patients with unilateral Meniere s disease Caloric reactions and hearing thresholds were compared separately at several frequencies A topodiagnostic relationship between the cochlear and vestibular function was discovered. Using four qualitative categories a significantly high correlation was obtained between the basal hearing loss (4 000 Hz) and unilateral weakness whereas no correlation was obtained when evaluating the more apical hearing loss (lower frequencies 250–1 000 Hz) A normal caloric reaction can be reasonably expected in cases of unilateral Meniere s disease if the hearing loss is less than approx 40 dB HL at 4 000 Hz.

Meniere s disease is characterized by a triad of symptoms namely unilateral sensoneural hearing loss tinnitus and paroxysmal vertigo It can be defined as a combined disturbance of the cochlear and vestibular functional systems Despite the common incidence of functional disturbances in both these systems a relationship between cochlear and vestibular function tests in Meniere s disease is scarcely known Enander & Stahle (1969) correlated the results of hearing loss defined as an average loss in the frequencies 500 1 000 and 2 000 Hz with the results of caloric testing in patients with Meniere s disease Their conclusion was that with increasing hearing loss a decreasing caloric reaction exists in the afflicted ear A topical correlation however between the cochlear and vestibular functional loss cannot be investigated by this method The purpose of the present study is to investigate the topodiagnostic relationship between the cochlear and vestibular function loss in Meniere s disease

PATIENTS

A total of 36 patients 19 male and 17 female, suffering from Meniere s disease were examined The patients were 48 years old, on average the youngest being 18 and the oldest 72 years All patients had a unilateral sensoneural hearing loss and contralateral hearing thresholds corresponding to their ages Patients with bilateral Meniere s symptoms were excluded from this study because it is quite impossible to evaluate unilateral weakness by means of caloric testing in these cases. Patients with supplementary noise induced hearing loss or a hearing loss on the other ear of more than 40 dB HL at 4 000 Hz were also omitted from this study Onset of the malady (patient s history) was on average 2 years previously in 4 cases it was less than 3 months and in 8 cases more than 5 years previously The patients presented a homogeneous group on the basis of their histories and audiological testing All the patients exhibited total or incomplete recruitment but no tone decay in respect to retrocochlear function All the patients also had a greater frequency discrimination loss in the lower tones than in the higher tones (Brix 1977) This is characteristic of Meniere s disease

METHODS

Hearing tests

(*a*) The thresholds for pure tones (pure tone audiogram) were determined on the right and the left ear for the following frequencies 125 250 500 1 000 2 000 3 000 4 000 6 000 and

:ed caloric response on the ipsilateral nth is then probable when the hearing at 4 000 Hz extends over 40 dB

DISCUSSION

most reliable results in assessing cochlear vestibular damage were obtained by rating the pure tone thresholds at 4 000 Hz g our qualitative criteria. The evaluation gher frequencies such as the more basally ted 8 000 Hz is not applicable because of tional negative factors such as presbya-s for example

rom the schematic drawing (Fig. 6) show the anatomic relationship in the inner ear can make the following topodiagnostic as nptions If apical damage is combined with ere damage in the basal part of the coch (e g 4 000 Hz) then it is very likely that the acent part of the labyrinth will also be naged On the other hand if there is only cally located cochlear damage without ex sive basal cochlear damage then the ves-llar labyrinth will not necessarily be af ted

This topodiagnostic relationship corre onds to the results obtained in experimental thogenesis of hydrops (Kimura, 1976). Ki-ura showed that inducing hydrops in guinea s resulted in atrophy of sensorineural ele nts in the apical part of the cochlea, where- the vestibular sensory cells were rarely af cted. Enander & Stahle (1969) obtained a latively low correlation (r=0.31 5% sign) cause hearing los wa determined by aver ing only the lower and middle frequencies a later study Stahle (1976) added an addi-onal frequency (3 000 Hz) to the averaging d thus obtained a higher correlation (=0 9) etween the degree of hearing loss and caloric npairment The purpose of our study was ot however to repeat and to verify what nander & Stahle have already done namely to demonstrate that increasing hearing loss in Meniere's disease is combined with an increasingly reduced caloric response We attempted rather to demonstrate a topodiagnostic relationship between cochlear and vestibular damage Additionally we wanted to demonstrate that a normal or reduced caloric reaction could be predicted by means of a pure tone audiogram.

ZUSAMMENFASSUNG

An einer Stichprobe von 36 Patienten mit einseitigem Morbus Meniere ist die cochleäre und vestibuläre Funktionstüchtigkeit untersucht worden. Die Kalorisationsantworten sind mit den Hörschwellen der verschiedenen Frequenzen separat verglichen worden. Zwischen dem cochleären und dem vestibulären Funktionssystem ist ein topodiagnostischer Zusammenhang aufgefunden worden. Bei Verwendung von vier qualitativen Kategorien ergab sich zwischen dem basalen Hörverlust (4 000 Hz) und der einseitigen Untererregbarkeit eine hohe signifikante Korrelation, während die Auswertung der mehr apikalen Hörverlustschwellen (250–1 000 Hz) keine Zusammenhänge zeigte. In Fällen von einseitigem Morbus Meniere kann eine normale kalorische Erregbarkeit mit großer Wahrscheinlichkeit dann erwartet werden wenn der Hörverlust bei 4 000 Hz kleiner als 40 dB HI ist

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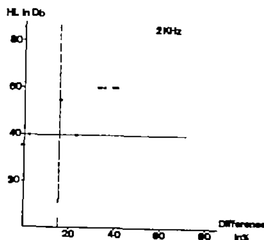


Fig 4 Hearing loss at 2 kHz (*ordinate*) against uni lateral weakness (*abscissa*) A weak correlation between the hearing loss and the caloric reaction is obtained

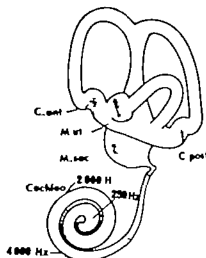


Fig 6 Schematic drawing of the inner ear showing the topographic relationship between the cochlear and the vestibular labyrinth

between the two labyrinths is defined as a pathological reaction

RESULTS

Pure tone audiogram and caloric reaction

The individual results of hearing losses for the frequencies of 250 500 1 000 2 000 and 4 000 Hz were compared directly with the results of the caloric reaction (reduced response in %) and are tabulated in Figs 1–5 It is evident that no clear correlation exists between hearing loss and caloric reaction for the frequencies of 250 500 and 1 000 Hz Since a differing contra lateral caloric reaction of up to 15% is defined as normal the caloric reaction can thus be divided qualitatively into normal caloric reac tions (15%) and reduced caloric responses (more than 15% difference) We therefore transformed all our data into qualitative cri teria. First we divided the hearing losses into a lesser (<40 dB HL) and into a greater range (>40 dB HL) and then we tabulated the calor ic response into a normal and a reduced re- sponse category Thus it became possible to calculate correlations between the hearing loss and the caloric response for each frequency No significant correlations were obtained for the frequencies of 250 500 and 1 000 Hz. This means that a reduced caloric response is some- times obtained irrespective of the degree of hearing loss in the above frequencies When a moderate or severe hearing loss exists in these frequencies one cannot predict the results of caloric testing by means of pure tone thresh- olds The first correlation was recognized at a frequency of 2 000 Hz where $r=0.4$ (2% sign). A substantial correlation however between the hearing loss and caloric reaction was ob- tained at 4 000 Hz Here the correlation was $r=0.85$ (1% sign) an extraordinarily high cor relation for biological data. This means that in cases of unilateral Meniere s disease one can expect a normal caloric reaction when the hearing loss is less than 40 dB at 4 000 Hz. A

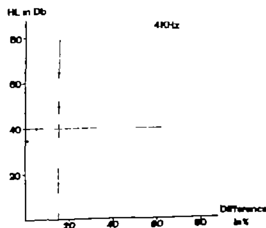


Fig 5 A high correlation is obtained between the hearing loss at 4 kHz (*ordinate*) and the caloric unilateral weak ness (*abscissa*)

Table I

Patient no	Age	Sex	Endocrine insufficiencies	GH Basal (pmol/l)	GH Peak after TRH (pmol/l)	Prolactin Basal (µg/l)	Prolactin Peak after TRH (µg/l)
1	52	♀		350	1 850	10	86
2	60	♂		2 900	4 200	6	30
3	36	♂	LH FSH	950	1 470	15	52
4	22	♀	LH FSH	4 200	6 800	22	42
5	34	♂	LH FSH	000	10 295	24	65
6	22	♂	LH FSH	1 725	004	52	77
7	34	♀	LH FSH	Normal	No increment	166	160
8	4	♀	LH FSH	Normal	No increment	260	282
9	73	♀	LH FSH ACTH TSH	Normal	N increment	42	62
10	64	♂	LH FSH ACTH TSH	Normal	N increment	11	30

Preoperative growth hormone (GH) and prolactin (PRL) levels in 10 patients with pituitary tumours later placed in organ culture. Basal levels are means of two morning samples within 10 min before intravenous injection of 200 µg TRH. Peak values after TRH were in all instances reached 30 min after the injection. Normal range for GH 12–433 pmol/l, for PRL <25 µg/l.

tion of Hanks BSS (Balanced Salt Solution) Under strict sterile conditions it was then divided into pieces not exceeding 1×1×1 mm. In tumours with necrotic parts only firm, solid areas were used for the culture. From each adenoma selected parts were immediately fixed in 3% glutaraldehyde in 0 133 M sodium phosphate buffer (pH 7 4) to preserve the original structures of the tumour. The number of pieces obtained from each adenoma exceeded 17 in all instances but frequently 20–40 separate pieces of the tumour could be kept in culture (Table II).

The culture medium varied slightly: most of the in vitro specimens were placed in a solution of Neuman & Tytell's serumless medium supplemented with 10% fetal calf serum (FCS) and 1% *l*-glutamine but other media were also used. Medium 199 (M 199) supplemented with 10% FCS and 1% *l*-glutamine. Trowell's T8 supplemented with 20% FCS and 1% *l*-glutamine and Neuman & Tytell's serumless medium supplemented with 10% horse serum. Antibiotics and antimycotics were not added even when the culture time was extended to one month.

Table II

Patient no	Endocrinologic type of tumour	No of specimens	Days in culture	Type of medium
1	GH	18	4–32	N & T
	GH	48	9–14	T 8 & M 199
3	GH	35	3–28	M 199
4	GH	26	7–32	N & T
5	GH	4	5–16	N & T
6	GH	4	3–31	M 199
7	PRL	30	3–16	T 8
8	PRL	18	7–14	N & T
9	PRL	18	1 14	N & T
10	No secretion of GH or PRL			

Summarizing the material used for organ culture. GH = growth hormone producing adenoma. PRL = prolactin-producing adenoma. Organ culture media used: N & T Neuman & Tytell' serumless medium. T8 Trowells T8. M199 Medium no. 199 (for details see Methods).

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IN VITRO PRESERVATION OF HUMAN PITUITARY TUMOURS IN ORGANOTYPIC DIFFERENTIATION

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(Received February 14 1979)

Abstract Three types of human pituitary adenomas—growth hormone producing prolactin producing as well as endocrinologically inactive ('chromophobe) tumours—were explanted to an in vitro system for organ culture After one month surviving hypophyseal cells demonstrated preserved hormone activity and no dedifferentiation of cell characteristics During the first weeks in culture close similarity existed between n vivo and in vitro conditions with regard to cell survival/cell morphology and physiological function/hormone release

Human pituitary tumours are frequently classified as such with hypersecretion of one or several of the pituitary hormones and others that are hormonally inactive It is still unclear whether these tumours are autonomous adenomas originating from the pituitary or in certain instances the results of primary suprapituitary disorders

To elucidate this an in vitro system for organ culture of the human pituitary gland offers the advantage of allowing studies of normal hypophyseal cells as well as of pituitary adenomas The cells can be maintained in an organotypic differentiation and when tissue architecture is retained—cell and tissue relations are normal—the situation is comparable to conditions in vivo and allows experimental manipulations of these characteristics In addition the effect of regulatory endogenous factors can be excluded

The present paper evaluates an organ culture system in which growth hormone (GH) and prolactin (PRL) producing human adenomas have been studied with light and electron microscopy as well as with hormonal analyses

MATERIAL AND METHODS

Patients

Pituitary tissue were obtained from 10 patients 5 men and 5 women (22–75 years of age) Six of them had GH producing adenomas and had acromegaly (nos 1–6) 3 prolactinomas (nos 7–9) while one (no 6) demonstrated hypersecretion of both hormones In one (no. 10) the tumour was hormonally inactive Table I lists endocrine insufficiences found at the preoperative evaluation Basal GH and PRL levels as well as the hormonal response to TRH were tested in all patients before surgery 200 μg of thyrotropin-releasing hormone (TRH) was given intravenously and GH and PRL analysed at −10 0 20 and 60 minutes thereafter The 6 patients with acromegaly showed a typical GH increment after TRH while in those with normal GH secretion there was no such response

The 3 patients with prolactinomas demonstrated no or blunted PRL responses to TRH while those 7 with normal basal prolactin levels (nos 1–6 and no 10) showed a clear PRL responsiveness to TRH

The transantro-(transmaxillary)-sphenoidal approach (Hamberger 1964) to the pituitary gland was used in 9 patients the transfrontal approach in patient no 6

Culture technique

Following surgical removal each pituitary adenoma was immediately placed in a solu-



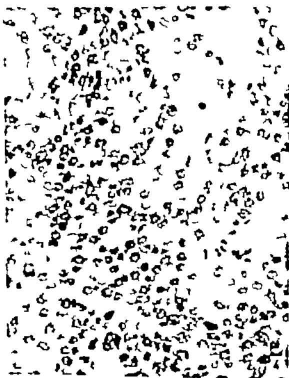






Fig. 3. LM. GH producing adenoma cultured in vitro for 3 days (patient 1). Groups of cells are arranged around lumens giving a glandular like appearance of tissue morphology. × 420.

Fig. 4. LM. The same adenoma tissue as in Fig. 3 but fixed immediately after surgical removal. Homogeneous arrangement of pituitary cells can be seen. × 420.

Fig. 5. LM. GH producing tumour cultured for 31 days in vitro (patient 6). Surviving cells in the periphery of the specimen. Central necrosis. × 420.

Fig. 6. Electron microscopy (EM). Detail from a prolactin producing cell cultured for 14 days in vitro showing preserved ultrastructure (patient 7). × 16 800.



Fig 1 Light microscopy (LM). GH-producing adenoma (patient 5) cultured for 16 days in vitro Central necrosis but cells adjacent to the periphery of the specimen are morphologically intact ×420



Fig 2 LM GH-producing tumour cultured in vitro for 14 days Small specimen without central necrosis (patient 7) ×180

The pituitary specimens were cultured either in culture dishes containing maximal humidity the medium (0 2 ml) being renewed every 2–3 days or in culture flasks containing 5 ml of the medium which was renewed after 7 days Hormone samples were obtained either by changing the culture medium as a whole (0.2 ml) or by drawing 0 2–0 5 ml from the culture flask The oxygen supply of the specimens took place via diffusion into the medium The organ cultures were kept at a constant temperature of +37°±0.2°C in air atmosphere

Hormone analyses

GH was measured by a double antibody radioimmunoassay technique according to Cerasi et al (1966) PRL was determined using a radioimmunoassay kit (CEA IRE SORIN)

Morphologic documentation

Following varying periods in organ culture specimens were taken for morphological prep-







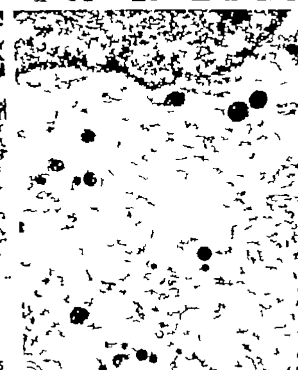


Fig. 3. LM. GH-producing adenoma cultured in vitro for 23 days (patient 1). Groups of cells are arranged around lumina giving a glandular-like appearance of tissue morphology. × 420.

Fig. 4. LM. The same adenoma tissue as in Fig. 3 but fixed immediately after [illegible] removal. Homogeneous arrangement of pituita[illegible] seen. × 420.

Fig. 5. LM. GH-producing tumour cultured for 31 days in vitro (patient 6). Surviving cells in the periphery of the specimen. Central necrosis. × 420.

Fig. 6. Electron microscopy (EM). Detail from prolactin producing cell cultured for 14 days in vitro showing preserved ultrastructure (patient 7). × 16 800.

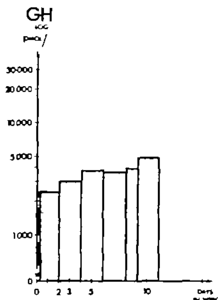


Diagram 1 GH production in vitro for 11 days (patient 1)

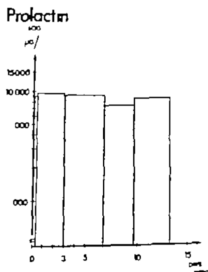


Diagram 3 PRL producing specimen cultured in vitro for 14 days (patient 8)

aration They were immersed with 3% glutaraldehyde in 0 133 M sodium phosphate buffer (pH 7 4) at +4 C for at least 2 hours Following rinsing in buffer for at least 2 hours the specimens were post fixed in 1% osmic tetroxide in Veronal acetate buffer (pH 7 4) dehydrated in increasing concentrations of alcohol and embedded in Epon mixture The entire material—more than 300 specimens—was sectioned for light microscopy and stained with toluidine blue Selected specimens were prepared for transmission electron microscopy (staining uranyl acetate and lead citrate)

RESULTS

General considerations

Specimens in organ culture were followed daily and a large part of the material was regularly photographed If cell degeneration had started the whole specimen appeared more translucent and less compact than living pituitary explants which have an opaque appearance Although antibiotics–antimycotics were not added bacterial contamination was very rare despite the medium in some specimens being changed 10–15 times during

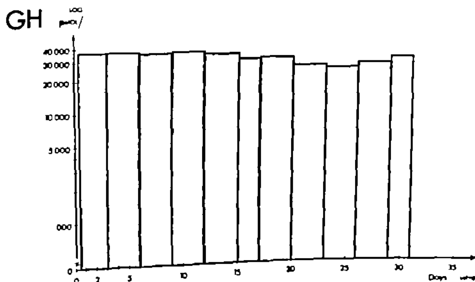


Diagram 2 High GH production in vitro for one month (patient 4)

e culture period Different organ culture edia were used though as indicated by cell irvival and hormone production they did not ary in their capacity to act as a nutrient

Tissue pieces exceeding ½–1 mm in the three imensions frequently showed central necrosis fter approximately one day while the re-maining parts appeared normal after 2–3 eeks (Fig. 1) Smaller specimens had intact tructures throughout (Fig. 2) A dense cell-ich adenoma could be transformed into a landular-like structure through slight necrosis f a few cells in the centre of groups of cells hereby giving the appearance of a more glan-dular configuration (Fig. 3) The control mate-ial fixed immediately after surgical removal of he tumour was, in general devoid of such structures (Fig. 4)

If cell degeneration started in small tissue fragments this occurred in the outer (lateral) regions of the adenoma in organ culture while the inner parts showed normal cells The opposite form of cell degeneration was found in the large pieces of adenoma tissue There was no difference in morphologic preservation of the organ cultures between culture dishes and culture flasks with increasing length of time in vitro Adenomas were explanted from subjects with varying age and size of the tu-mour (adenoma/microadenoma) but no con sistent difference occurred among the speci-mens concerning cell survival in the organ cul-ture system

GH-producing adenomas

For all tissue pieces cultured in vitro a number of controls were used for ultrastruc tural morphology at the time of explantation Neither dedifferentiation of pituitary cells nor growth of the adenoma occurred with increas-ing times in vitro The GH producing cells were followed for one day – one month in vitro both concerning morphological preserva-tion and GH activity These were stable for at least 3 weeks and sections through such specimens showed well preserved cell mor phology (Diagram 1) However thereafter cell degeneration started and, at one month sometimes (but not always) only ½–⅓ of all cells in a section through the specimen ap-peared healthy (Fig. 5) There were exceptions where specimens demonstrated rather pre served morphology and an unchanged high hormone production (Diagram 2)

The hormone production into the medium was measured continuously and correlated well with the endocrinologic activity found in vivo (patient no 1 Diagram 1 patient no 4 Diagram 2)

GH-producing cells were sometimes ob-served secreting granules into the extracellular space of the organ cultures In degenerating GH-cells granules became liberated and seemed morphologically preserved mutually while the remaining part of the cell showed severe degeneration The mature secretory granules in vitro were electron dense and sur rounded by a membrane There was a good correlation between the distribution of gran-ules in cells of organ culture and in specimens taken for fixation immediately after surgical removal Microvilli did not develop on the sur face of the cells even during long-term culture.

PRL-producing adenomas

The general principles for organ culture of these specimens were the same as for GH producing adenomas Tissue pieces were studied for 1–21 days in vitro and demon-strated cell survival and hormone release into the culture medium (Fig. 6 Diagram 3) Dedif ferentiation did not occur All specimens were photographed daily and in no case did growth of the adenoma occur

Endocrinologically inactive tissue

Specimens were cultured in vitro for 2–11 days Morphological analysis revealed that during this time the tumours followed the same general principles for cell survival as GH and PRL-producing adenomas The culture medium was analysed for GH and PRL contents These were below measurable values with the present assay methods

DISCUSSION

Tumours developing from pituitary paren chyma can be classified on morphological and endocrinological basis Functional analysis correlating anatomical and physiological characteristics allow for cellular identification

The present investigation revealed that human pituitary adenoma tissue can be kept in organ culture for at least one month The cultured tissue maintained a structural state comparable to that found in fresh material Both GH and PRL secreting adenomas re acted in a rather similar way to the in vitro conditions independent of the three types of culture media used

Only a limited number of studies have been reported on the culturing of human pituitary adenomas in vitro during which morphology and hormonal secretory activity have been concomitantly followed Thus Peillon et al (1975) were able to maintain high GH secre tion in vitro from somatotrophic adenomas for 2 weeks with a certain degree of correlation remaining between morphological and bio-chemical results In most of such studies however the pituitary tissue was either homo-genized (Guyda, 1975 Matsukura et al 1977) or trypsinized (Batzdorf et al 1971) to achieve suspension cultures prior to experimental manipulation Goodyer et al (1977) kept fetal human pituitary cells in vitro up to 6 weeks both in monolayer and organ cultures Al though the cells of adenomas in culture like those of normal pituitary and intact adenomas are not bound by junctional complexes the separation by mechanical or chemical/enzy matic methods may influence the characteris-tics of the plasma membrane and the extrusion of granules In the present study the interrela-tionship between cell structure and hormone secretion could be upheld

The pituitary tissue did not grow during the investigation period This is in agreement with the biological behaviour of the tumour in vivo with slow growth during a period of several years

During the beginning of the culture period a high degree of stability was maintained in the in vitro system Extrusion of granules was observed by electron microscopy after 3 weeks in organ culture from GH and PRL producing cells with well preserved ultra-structure thereby documenting active hor mone production and release from these cells.

In the present organ culture system, no de-differentiation occurred with regard to ultra-structural characteristics within the investi-gated period of one month The outlines of cells examined in thin sections are affected by the way in which they were preserved in vitro and prepared for electron microscopy See man & Dmochowski (1975) reported that the ultrastructure of human tumour cells in vitro scraped or trypsinized from monolayers, lose most of their intercellular connections with retraction of the cytoplasm Therefore this may result in a redistribution of cytoplasmic organelles In contrast the anatomy of cells in organ culture fixed in the same way as biopsy tissue in theory is satisfactorily preserved

Hormone production into the medium oc curred throughout the culture period This indicates optimal conditions in the in vitro system and compares well with the in vivo milieu concerning the two investigated basic functions cell survival/cell morphology and hormone synthesis and release (physiological function) Good agreement existed between the preoperative endocrine activity and hor mone production in vitro

Studies are now in progress to analyse how the release of GH and PRL can be regulated in this in vitro system and correlated with ultrastructural characteristics

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Drei Arten von menschlichen Hypophysenadenomen – zuwachshormonproduzierende prolactinproduzierende

...wohl ... endokrinologisch inaktive („chromophobe") Tumore – wurden in ein *in-vitro*-System für Organkulturen implantiert. Nach einem Monat wiesen die überlebenden Hypophysenzellen beibehaltene Hormonwirksamkeit und keine Dedifferenzierung der Zellencharakteristik auf. Während der ersten Wochen in den Kulturen existierte ein guter Parallelismus zwischen den *in-vivo*- und *in-vitro*-Zuständen, was das Überleben der Zellen/Zellenmorphologie und die physiologische Funktion/Freigebung der Hormone betrifft.

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ULTRASTRUCTURAL FINDINGS OF THE NASAL MUCOSA OF OZAENA" IN ATROPHIC RHINITIS

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(Received December 11 1978)

Abstract Alterations in the nasal mucous layer of atrophic rhinitis *Ozaena*" patients have been investigated The vast majority (99%) of these patients were women Morphological findings in healthy nasal mucosa demonstrated the different functional stages of the glandular tissue cells with the healthy epithelium On the other hand nasal mucosal material taken from the patients displayed prominent epithelial deterioration decrease in and loss of cilia, increase in goblet cells with squamous epithelial metaplasia. A decrease in secretory granules and membrane deterioration in the apical region of the secretory cells was also clearly visible It is possible to postulate that the ultrastructural changes seen in the secretory and storage cycles of glandular tissue of the nasal mucosa from patients seem to arise as a reaction to the superficial epithelial deterioration of the nasal mucosa together with the resulting deterioration of physiological conditions

Ozaena is a frightful disease which upsets the whole life of a patient without being lethal Mucosal atrophy and decrease in secretion causes a crust formation which fills up the nasal cavity obstructs the nasal canal and discharges on offensive odour which disturbs the patients

The first information on Ozaena was published by Kahler in 1921 and Lautenschlager gave a detailed description of this disease in 1927

Roy (1915) reported that he had not encountered any cases of Ozaena among negroes nor have any Ozaena cases been reported in Arabs Ozaena mainly occurs in members of the white and the yellow race The disease is encountered in Spain Greece Poland Russia and mainly Turkey with an incidence rate of 2–4% Ozaena still constitutes one of the major problems of rhinology despite the fact that much has been said and written about its etiology and its management

Several theories regarding the etiology of Ozaena have been advanced. (*a*) the constitutional heredity theory (*b*) the infection theory (*c*) the neurovegetative theory (*d*) the endocrine theory However since none of these theories has been validated medical and surgical treatment has up to now only been based on the symptoms

In addition to the clinical observations, there are some scanning and transmission electron microscopic findings which are mentioned in two studies (Mygind et al 1974), but these observations have been limited to the epithelial metaplasia in patients

In the present study we were especially interested in evaluating the ultrastructural findings concerning the glands of the nasal mucosa, as well as epithelial modifications

MATERIAL AND METHOD

We have carried out various investigations on Ozaena patients over a period of 20 years and we have observed that the vast majority of these patients are women for this reason the material utilized in this investigation was obtained from women patients

Mucosal samples of the inferior part of the conchae were obtained from 10 women—



Fig. 1 (a, b). Electronmicrograph of the nasal mucosa from the patient with atrophic rhinitis. Note the epithelial metaplasia of the columnar pseudostratified epithelium ( ) into squamous stratified epithelium (*b*). Tonofilaments *T* and desmosome attachments (*D*) are seen in (*b*).

Fig. Electronmicrograph of the concha from patient with Ozaena. ( ) Note the epithelial deterioration and loss of cellular membranes and microvilli in the apical region of the epithelial cells. Also the partially maintained cilia (*cl*) and haemorrhagic appearance are evident. (*b*) Epithelial disturbance and groups of cilia (*Cl*) are demonstrable at higher magnification.

Fig. 3 Electronmicrograph of the nasal mucosa of the concha region taken from patient with Ozaena. Note the increase in number of goblet cells varying in their secretory cycles.

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Mucosal samples of the inferior part of the conchae were obtained from 10 women—

normal and 8 suffering from Ozaena—under local anaesthesia, and were investigated electron microscopically with the aim of reaching a conclusion by comparing the normal and diseased mucous membranes.

Biopsy specimens of a size of 2 mm were fixed 1 hour at 4°C with buffered 1% osmium tetroxide (pH 7 3)

After dehydration in acetone the blocks were embedded in Vestopal W Ultrathin sections were double stained with uranyl acetate and lead citrate and studied by JEOL 100 C electron microscope

RESULTS

Our ultrastructural findings in healthy mucosa reflected different functional secretory phases with their granular endoplasmic reticulum (GER) and the Golgi complexes (Figs 4 and 6) Some of the glandular cells had an organelle poor lucid cytoplasm whereas some other cells were rich in secretory granules.

In material obtained from atrophic rhinitis patients, the concha mucosa showed epithelial destruction (Fig. 2a b) decrease and loss of cilia, and increase in goblet cells (Fig 3) as well as in squamous epithelial metaplasia (Fig. 1a b) The glandular secretory cells are seen to have very active Golgi complexes but secretory granules were decreased at their apical region with varying number shape and density In the apical parts of the cells facing the lumen destruction of the cytoplasmic membrane and depletion and even disappearance of microvilli have been observed (Figs 5 and 7)

DISCUSSION

As it is known from the literature knowledge various nasal mucosal changes of patients with Ozaena drew the attention The most important of these changes were the transformation of the characteristic pseudostratified columnar epithelium into a squamous epithelium and the disturbance of the physiological medium Moreover the deficiency of a protease inhibitor in the nasal secretion of atrophic rhinitis cases reported by Reichert & Hochstrasser (1971) might play an important role in the pathogenesis of the disease

In addition to the epithelial metaplasia, our ultrastructural findings concerning the alterations of the glandular cells of the nasal mucosa material taken from the patients with Ozaena also showed some disturbance in the secretory stages in the glandular tissue

In the light of these findings it is possible to suggest that the secretory and storage processes of the nasal glandular tissue taken from the patients with Ozaena are disturbed by alterations of physiological conditions caused by superficial epithelial transformations in these patients

ZUSAMMENFASSUNG

Um in die Ätiologie der atrophischen Rhinitis ein Licht zu bringen, wurden elektronenmikroskopische Untersuchungen durchgeführt. Es wurde festgestellt, daß bei Patienten, die an atrophischer Rhinitis erkrankten unter entsprechenden physiologischen Bedingungen, an der Nasenschleimhaut Oberflächenveränderungen vorkamen. Besonders die Sekretfunktion der Nasendrüsen wurde auf ultrastruktureller Ebene verzeichnet.

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Fig 6 Electronmicrograph of the healthy mucosa of the conchae Note the secretory granules (*Sg*) and microvilli (*Mv*) in the apical region of the glandular cells Also active Golgi complexes (*Gc*) are seen.

Fig 7 Electronmicrograph of the conchae of atrophic rhinitis patient Note the decrease in secretory granules (*Sg*) and loss of microvilli with membrane deterioration of the apical cytoplasmic membrane Also active Golgi complexes (*Gc*) are seen *L* lumen

ing PBS by shaking The material was ed by adding Azur II nuclear stain in -Grünwald/Giemsa, dissolved in PBS Af adding a coverglass the wet specimens examined directly at 1250× magnifica- using oil immersion and differential inter nce contrast microscopy (DIC)

he discrimination of normal squamous epi al cells from metaplastic or immature mous cells took place according to routine logical criteria including the evaluation of relative sizes of nucleus and cytoplasm to the difficulty of distinguishing between aplastic and immature cells in the indi- al cases all these cells were designated etaplastic for the purpose of this study

he amount of bacterial adherence to about squamous epithelial cells was registered relative number of ciliated cells was also ed as well as the presence of any bacterial chment.

he number of granulocytes in each field of ion was registered In the brushings from adenoid surface we also noted the pres- e of keratin flakes

Nine of the adenoids were fixated in forma- (7%) embedded in paraffin wax and cut thin sections Staining for ultraviolet orescence microscopy was made with acri- e orange at pH ...7 The presence of bac a on the adenoid surface and within the ue was studied at 1..50× magnification otography confirmed the observations

RESULTS

great number of mature normal squamous ithelial cells were seen in the secretions ob- ned from the adenoid surface Ciliated epi- thal cells were present in 5 patients In all ses large numbers of granulocytes were esent There was an average of 10–30 bac ria attached to each squamous epithelial cell In the brushings from the soft palate large mbers of normal squamous epithelial cells ere found The average number of attached cteria corresponded to the number of bac

Table I *Average number of bacteria attached to squamous epithelial cells in samples from three sites in the nasopharynx*

Patient no.	Adenoid	Secretion	Soft palate
1	0	33	9
2	0	16	15
3	0 5	18	6
4	0	46	11
5	0	24	35
6	0 5	18	16
7	0	15	12
8	1.4	20	13
9	2	42	20
10	0	13	3 4

teria adhering to squamous epithelial cells in the secretions covering the adenoid surface (Table I) Ciliated cells were seen in all pa tients but one Granulocytes were much less frequent than in the secretions covering the adenoid

Ciliated epithelial cells were found in brush- ings from all adenoids. In 8 cases the yield also contained large amounts of metaplastic squamous epithelial cells In 7 of these 8 cases keratinization was observed Normal squa- mous epithelial cells on the other hand were found only very occasionally and then in samples from 4 patients In these 4 cases bacteria were generally seen to attach to these cells Attachment to metaplastic squamous epithelial cells from the adenoid surface was seen in 1 patient Singular large coccoid bac teria were occasionally seen to adhere to kera- tin flakes In only one case did a bacterium ap- pear to be attached to a ciliated cylindrical cell There were fewer granulocytes in the brushings from the adenoid surface than in the overlying layer of secretion

The mature normal squamous epithelial cell had a microridge-covered surface whereas the cells, designated metaplastic cells in this study showed a rough granular surface

In the sections of adenoid tissue bacterial attachment to the adenoid surface was seen very infrequently Thus in only one case were bacteria seen to adhere to the surface in cer

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BACTERIAL ADHERENCE TO EPITHELIAL CELLS IN THE NASOPHARYNX IN CHILDREN

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(Received February 9 1979)

Abstract The presence of attached bacteria to epithelial cells from the nasopharyngeal surface of the soft palate from the adenoid surface and from the secretions covering the adenoid was studied in 10 children undergoing adenoidectomy Large numbers of bacteria were seen to attach to mature normal squamous epithelial cells from the soft palate and in the secretions whereas attachment to adenoid epithelial cells was rare Using differential interference contrast microscopy bacteria-carrying epithelial cells were seen to have their surface covered by microridges characteristic of normal mature squamous epithelial cells Sections of adenoid tissue showed bacterial infiltration of adenoid tissue to be virtually non-existent in the patient group

Bacterial adherence to epithelial cells as a possible pathogenic factor in infection is a rapidly growing field of study In 1972 Gibbons & van Houte showed the cell and species specificity of various types of streptococci adhering to oral mucosal cells Further studies have shown that bacterial adherence is a relevant factor in the pathogenesis of bacterial infections (Svanborg Edén et al 1976) It has also been shown that cells from infection prone individuals may have a more pronounced tendency to attach to bacteria that *per se* are regarded as pathogenetic (Källenius & Winberg 1978)

An essential defence mechanism against the attachment of bacteria is IgA antibodies which are considered to aggregate bacteria and prevent their attachment to the mucosal cell surfaces (Williams & Gibbons 1972) The secretions themselves are also regarded as protective both because they constantly wash the mucosa and because of their molecular properties which may aid in preventing the penetration of antibody-coated bacteria to the mucosal surface (Magnusson 1978)

Numerous studies have shown the presence of stationary floras of pneumococci and *Haemophilus influenzae* in the nasopharynx, which makes the relevancy of bacterial attachment in this region an interesting study (Kamme et al 1971) Our study concerned the distribution of bacteria on epithelial cells in the nasopharynx in children The bacterial invasion of adenoid tissue was also examined. To facilitate this survey new techniques were developed using nuclear stains and differential interference contrast microscopy on unfixed cell specimens

MATERIAL AND METHODS

Ten children (age range 3–10 years) were examined in connection with adenoidectomy due to recurrent infections of the upper respiratory tract otosalpingitis and nasal obstruction

After intubation the soft palate was retracted and the secretions covering the adenoid surface collected Subsequently cells were brushed off the nasopharyngeal surface of the soft palate using a grooved metal hook

After removal the adenoid was washed and rinsed thoroughly with phosphate-buffered saline solution (PBS) Cell brushings were taken from several sites of the adenoid surface under a preparation microscope

All samples were suspended in PBS and washed three times by centrifugation at 1000 rpm for 8 minutes After pouring off the supernatant the pellet was resuspended in the re-

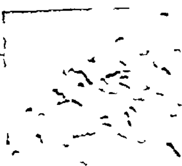


Fig. 1

thelial cells was very contrasting and impressive. The implication of these observations is that bacterial attachment in the nasopharynx is cell-specific and almost invariably engages only normal squamous epithelial cells on the mucosa or in the nasopharyngeal secretions.

A conspicuous characteristic of normal mature squamous epithelial cells, in addition to well known cytological criteria, was the presence of microridges on the surface of these cells. The metaplastic epithelial cells on the other hand had a rough granular surface. This difference in surface structure between normal and metaplastic squamous epithelial cell has previously been reported only in studies on epithelial cells with sweep electron microscopy (Rubio & Kranz 1976). The present study shows that this difference between the two types of epithelial cell is not merely a difference in morphology. The surface structure of the epithelium is also related to the phenomena of bacterial attachment (Fig. 1).

The presence of normal mature squamous epithelial cells together with small epithelial cells on the soft palate leads to the conclusion that at least some of the small cells were immature normal cells. Brush specimens from the adenoid surface contained keratin flakes and small squamous epithelial cells but only very occasional mature normal squamous epithelial cells with microridges. This indicates metaplasia of the columnar cells of the mucosal surface. However, the small cells from both locations are indistinguishable and hence for the purpose of this study have been designated as metaplastic.

A large number of bacteria, either attached to epithelial cells or free, were present in the secretions covering the adenoid surface or filling the crypts and glandular ducts. Only in a very few instances were bacteria seen adjacent to the cell surfaces of the adenoid or in the epithelial cell layer itself. In no cases were bacteria observed in the lymph follicles. This implies that the contact between bacteria and the adenoid mucosal surface is often less intimate than could be expected.

In this context it is also essential to point out that large numbers of granulocytes were present in the secretions containing the great majority of bacteria. Granulocytes were less frequent in brushings from the adenoid surface and even fewer in the material from the soft palate. Further, in sections of adenoid tissue, granulocytes were very sparse in the epithelial layer except in occasional cases showing signs of inflammatory reaction within the mucosa. Taken together, these observations imply that the inflammatory reaction, as judged by the presence and location of bacteria and granulocytes, generally takes place outside the adenoid, i.e. in the secretions overlying the mucosal surface of the adenoid. However, the reliability of this hypothesis must be weighed against the fact that none of the patients examined had a fulminant acute adenoiditis.

ZUSAMMENFASSUNG

Die Adhärenz von Bakterien an epitheliale Zellen der nasopharyngealen Oberfläche des weichen Gaumens und der Rachenmandel und an Zellen des die Rachenmandel überziehenden Sekrets wurde bei 10 Kindern untersucht, die einer Adenoidektomie unterzogen. Eine grosse Bakterienmenge war an Plattenepithelzellen des weichen Gaumens und des Sekretionsschleimes geheftet. Einen Adhärenz an die Oberfläche der Rachenmandel gab es dagegen kaum. Mit der Nomarski-Optik konnten wir

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Only in very rare instances were bacteria seen to attach to metaplastic epithelial cells or to keratin flakes the latter often with preserved cell configuration Bacterial attachment to ciliated epithelial cells was seen only on one occasion which however consisted of one bacterium apparently adhering to one single cell out of many hundreds examined. Thus the observation of large numbers of bacteria attached to normal squamous epi-

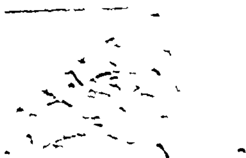


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belial cells was very contrasting and impressive The implication of these observations is that bacterial attachment in the nasopharynx is cell-specific and almost invariably engages only normal squamous epithelial cells on the mucosa or in the nasopharyngeal secretions

A conspicuous characteristic of normal mature squamous epithelial cells in addition to well known cytological criteria, was the presence of microridges on the surface of these cells The metaplastic epithelial cells on the other hand had a rough granular surface This difference in surface structure between normal and metaplastic squamous epithelial cells has previously been reported only in studies on epithelial cells with sweep electron microscopy (Rubio & Kranz, 1976) The present study shows that this difference between the two types of epithelial cell is not merely a difference in morphology The surface structure of the epithelium is also related to the phenomena of bacterial attachment (Fig 1)

The presence of normal mature squamous epithelial cells together with small epithelial cells on the soft palate leads to the conclusion that at least some of the small cells were immature normal cells Brush specimens from the adenoid surface contained keratin flakes and small squamous epithelial cells but only very occasional mature normal squamous epithelial cells with microridges This indicates metaplasia of the columnar cells of the mucosal surface However the small cells from both locations are indistinguishable and hence for the purpose of this study have been designated as metaplastic

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In this context, it is also essential to point out that large numbers of granulocytes were present in the secretions containing the great majority of bacteria. Granulocytes were less frequent in brushings from the adenoid surface and even fewer in the material from the soft palate Further in sections of adenoid tissue granulocytes were very sparse in the epithelial layer except in occasional cases showing signs of inflammatory reaction within the mucosa. Taken together these observations imply that the inflammatory reaction as judged by the presence and location of bacteria and granulocytes generally takes place outside the adenoid i.e in the *secretions over*lying the mucosal surface of *the adenoid* However the *reliability of this hypothesis* must be weighed against the fact that none of *the* patients examined had a fulminant acute adenoiditis

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feststellen dass die Oberflache der bakterientragenden Zellen mit Mikrofurchen versehen ist was fur normales reifes Plattenepithel charakteristisch ist. Untersuchungen von Schnittpraparaten der Rachenmandel zeigten dass das Vorkommen von bakterieller Infiltration der Rachenmandelschleimhaut selten ist.

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AMYLOIDOSIS OF THE LARYNX

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ract Amyloidosis of the larynx is rare disease unting for less than 1% of all benign laryngeal ours Three cases of this type of lesion are reported e of the vocal cord, one of the false vocal cord and of the subglottis and trachea. In 2 of the patients amyloidosis was localized, while the third was later d also to have an epipharyngeal solitary plasma- ma with amyloid deposits and in addition amyloi- s of the nasal cavity However the amyloidosis in this ent may still be regarded as being localized, as the cal examination and laboratory tests afforded no dence of generalized disease Amyloidosis of the nx may be manifested as localized tumour or as bse infiltration The symptoms and signs will of rse, depend on the site of the amyloid deposit When vocal cords are involved hoarseness may result, and s as the most prominent sign in the present cases The atment of laryngeal amyloidosis is primarily by endo- pic excision Amyloid substance has specific staining perties The Congo red reaction with green bire- gence in polarized light and Thioflavine BBU using orescence microscopy are regarded as the most re- ble staining reactions Electron microscopy has re- aled typical fibrillar structure of amyloid.

he pathogenesis of amyloidosis is unknown ie condition is characterized by extracellular eposits of a proteinaceous substance (Glen- er & Page 1976) Amyloidosis was first de cribed by Rokitansky in 1842 and the term amyloidosis was coined by Virchow in 1851 The first report of a patient with laryngeal amyloidosis was published by Burow & Neu- mann in 1875 since when several cases have een described In a review article McAlpine & Fuller (1964) collected 177 cases Another 17 have been reported from the Mayo Clinic (Ryan et al 1977) There are also many case reports (Stark & New 1949· Juselius & Ny- berg, 1960· Vieta & Ouraieb 1964 Heiner 1968 Ellachar & Lichtig 1970· Beasley 1971 Crifò 1973 Barnes & Zafar 1977)

To elucidate the pathogenesis and clinical behaviour of amyloidosis extensive studies have been carried out with the aid of for example electron microscopy and immuno-chemical techniques (Cohen 1967 Ellachar & Lichtig 1970· Franklin & Zucker Franklin 1972 Glenner et al 1972 Glenner & Terry 1974 Glenner & Page 1976 Kaiser Kupfer et al 1977) Amyloidosis can be either gen-eralized—and then primary or secondary—or localized The nature of amyloidosis is still an enigma, however

Amyloidosis accounts for less than 1% of all benign laryngeal 'tumours It occurs slightly more often in males and usually between the ages of 40 and 60 (Stark & New 1949· McAl-pine & Fuller 1964) According to some re ports the sites are in descending order of frequency the false vocal cords aryepiglottic folds and the subglottic region (Leroux Ro-bert, 1962 D'Arcy 1972) but the report from the Mayo Clinic (Ryan et al 1977) gives the vocal cords as the most common location

During the last 10 years 3 cases of laryngeal amyloidosis have been encountered in this hospital.

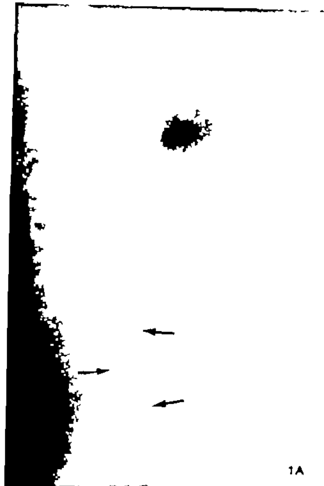
CASE REPORTS

Case 1 (Fig. 1 A, B)

History and clinical findings The patient, a woman now 77 years of age sought medical attention in 1966 for hoarseness from which



Fig 1 (Case I) (A) Conventional plain film lateral view Subglottic swelling with extension down into the upper trachea (*arrows*) (B) Microlaryngoscopy A diffuse subglottic swelling with intact overlying mucosa.



she had been suffering for the past year Laryngoscopic examination revealed a subglottic swelling especially posteriorly Histological examination of biopsy specimens yielded a diagnosis of amyloidosis A blood-cell count a bone marrow examination and liver and renal function tests disclosed no pathological features ESR was 38 mm Serum protein electrophoresis showed a slightly elevated gammaglobulin level Chest radiographs and ECG registrations were unremarkable Tongue and rectal biopsies disclosed no amyloid deposits

The patient was readmitted in 1967 because of slowly increasing inspiratory difficulty and excisions were performed

Ten years later there was again increasing inspiratory stridor Radiological examination of the larynx and trachea showed marked soft tissue swelling of the subglottis and the upper 3 cm of the trachea (Fig 1 A) The airway was restored by excisions through laryngo- and bronchoscopes (Fig 1 B) New laboratory tests including a fine needle biopsy of the abdominal fat showed no evidence of generalized amyloidosis

Pathological findings The histological examination of the excised tissue disclosed a loose connective tissue covered with ordinary

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Comment As the laboratory examinations ncluding the rectal and abdominal fat biop- ies showed normal conditions with no sign of generalized disease the amyloidosis must be regarded as being localized

Case 3 (Fig 3 A–C)

History and clinical findings The patient is a now 61 year-old man with a history of heavy cigarette smoking and abuse of alcohol From 1966 to 1968 he noticed increasing hoarseness Laryngoscopy (1968) showed a polypous swelling of the right vocal cord Histological examination of the excised tissue disclosed amyloidosis The ESR a blood-cell count and a bone marrow examination serum protein electrophoresis liver and renal function tests urography ECG chest radiography and other laboratory tests revealed no evidence of pathological conditions Cholecystography showed a solitary gall stone Radiography visualized an opaque maxillary sinus A Luc Caldwell operation disclosed a bilocular cyst Skin tongue and rectal biopsies were normal

In 1969 a tumour of the upper surface of the soft palate was diagnosed At histological ex amination a plasmacytoma with amyloid de- posits was identified Following radiotherapy (60 Gy) the tumour disappeared and there has been no sign of recurrence

In 1969 and 1971 repeated radiological ex aminations of the skull thorax chest and pel- vis showed no signs of myelomatosis

In 1972 there were increasing laryngeal symptoms, and the radiological examination and the microlaryngoscopy now showed a right glottic and subglottic tumour (Fig 3 A B) and more of the amyloid tissue was excised

In 1978 a polypous easily bleeding swelling of the right nasal cavity was noted and a his- tological diagnosis of amyloidosis was made A fine needle biopsy of abdominal fat did not show any evidence of a generalized disease

Pathological findings The initial laryngeal polypous lesion was covered with ordinary squamous epithelium The submucous con- nective tissue was diffusely infiltrated with an amorphous substance surrounded by leuko- cytes It stained with Congo red and showed

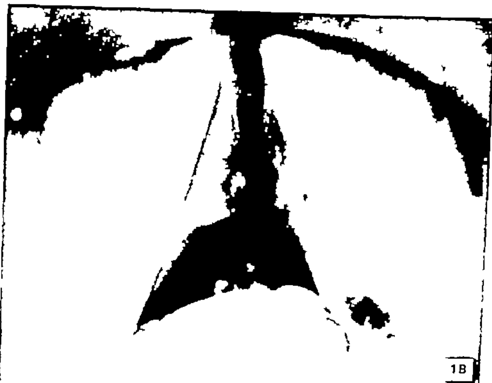


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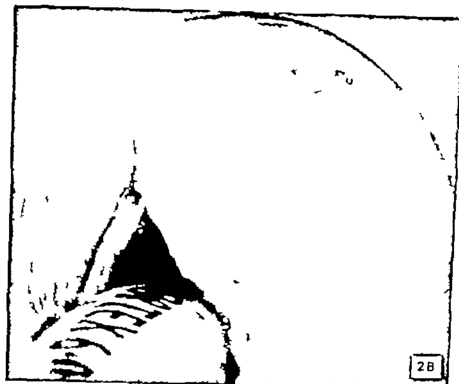
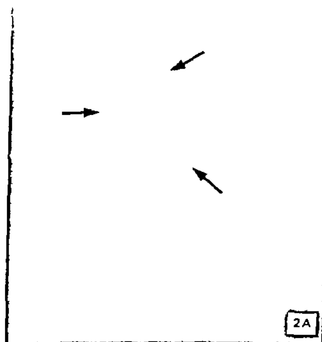


Fig 2 (Case) (A) Tomogram, a–p view Bulging of the right false vocal cord and aryepiglottic fold with obliteration of the right ventricle (*arro s*) (B) Microlaryngoscopy Smooth swelling of the right false vocal cord with intact over lying mucosa.



a typical green birefringence when viewed in polarizing light but did not stain with PTAH For the material examined later the histological findings were similar (Fig 3 C)

The biopsy of the nasopharyngeal tumour was covered with columnar epithelium The richly vascularized stroma was profusely infiltrated with atypical plasma cells An amorphous substance staining positively with Congo red was also present

The nasal polyp showed marked oedema with amyloid deposits

Comment As the rectal tongue skin and abdominal fat biopsies were negative and the other laboratory tests disclosed no generalized disease the amyloidosis was considered to be primary being localized to the upper respiratory tract It might be objected that there was also a solitary plasmacytoma but there was no myelomatosis

DISCUSSION

After more than a century s investigation the nature of the unique proteinaceous amyloid substance remains obscure Electron micrographs show all amyloid to have the same basic fibrillar structure with rigid and unbranched fibrils measuring 50–150 Å in diameter (Kyle & Bayrd 1975 Glenner & Page 1976) The length of the fibrils has been estimated at about 8000 Å (Kyle & Bayrd 1975) Chemical analysis has distinguished three chemical types of amyloid one of immuno-



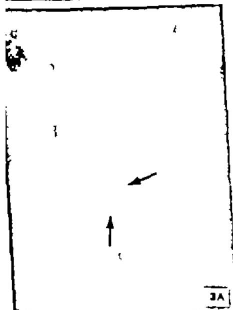




Fig. 3 (Case 3). (A) Laryngogram, p-a view. Tumour of the right vocal cord with subglottic extension. (B) Microlaryngoscopy. Swelling of the right vocal cord with intact overlying mucosa. (C) Photomicrograph. Infiltration of an amorphous substance (amyloid), staining positively with Congo red, in the submucosa with intact overlying epithelium (× 370). *Inset*: Brilliant green birefringence in polarized light (× 200).

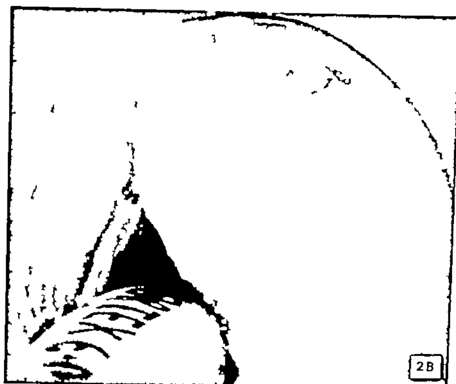
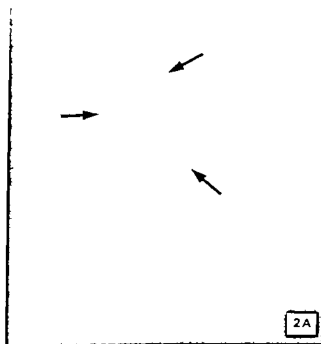


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globulin origin one of unknown origin and the third apudamyloid (Pearse et al 1972 Glenner & Page 1976 Barnes & Zafar 1977) The amyloid fibrils associated with generalized primary amyloidosis and some forms of localized amyloidosis and those associated with plasma-cell dyscrasia are derived primarily from the aminoterminal (variable) region of a light chain of a homogeneous immunoglobulin (Glenner & Terry 1974 Glenner & Page 1976) The amyloid of unknown origin has fibrils not deriving from immunoglobulin this type of amyloid is also found in secondary amyloidosis and the familial Mediterranean fever The third type apudamyloid is associated with endocrine polypeptide tumours (Pearse et al 1972)

Congo red the most commonly used staining reaction for amyloid gives a bright red colour In polarizing light an apple-green birefringence is obtained These tinctorial and polarization properties are due to the presence of β-pleated sheet fibrils (Glenner et al 1972) The presence of these fibrils is revealed by low angle X ray diffraction which is the third principal method (after light and electron microscopy) for identifying the amyloid substance (Kyle & Bayrd 1975) The Congo red reaction is sensitive and false positives and negatives are rare Evidently still more sensitive however is Phorwhite BBU with even fewer false staining reactions (Waldrop et al 1973 Glenner & Page 1976) Crystal violet gives a positive metachromatic reaction A new potassium permanganate technique for distinguishing between the chemical types of amyloid has been introduced by Wright et al (1977)

The majority of the numerous clinicopathological classifications of amyloidosis are modifications of that published by Symmers in 1956 (1) generalized secondary amyloidosis (amyloidosis associated with an identifiable predisposing disease) (2) generalized primary amyloidosis (no identifiable predisposing disease) and (3) localized amyloidosis The larynx is rarely involved in generalized secondary amyloidosis and infrequently in generalized primary amyloidosis The larynx is however the usual site for amyloidosis of the respiratory tract

Amyloidosis within the larynx occurs in two forms one of them tumour-like and the other displaying diffuse infiltration The manifestations of the disease will of course depend on the site of the amyloid deposit involvement of the vocal cords will result in hoarseness subglottic involvement often leads to increasing inspiratory difficulty and in the case of supraglottic deposits the symptoms prompting the patient to seek medical attention may be more diffuse The diagnosis is obtained on the basis of laryngoscopic and biopsy findings Further information on the extent of the lesion may be provided by a radiological examination

Four patterns of extracellular deposit of amyloid in the larynx may be distinguished. (1) amorphous random masses (2) in vessel walls (3) in basement membranes of the seromucinous glands and (4) as hyaline rings in adipose tissue (Barnes & Zafar 1977). The amyloid tumour is of course not an actual neoplasm but rather a localized tumour-like swelling produced by the presence of the amyloid deposit in the submucosal tissue

Two cases (cases 1 and 2) are both examples of localized amyloidosis The third case (case 3) is questionable but is probably also to be regarded as localized amyloidosis of the respiratory tract since fairly exhaustive investigation established no evidence of generalized disease notwithstanding the presence of a solitary nasopharyngeal plasmacytoma.

In a patient with amyloidosis it is necessary to eliminate the possibility of a generalized disease Among the laboratory tests and other methods that should be used to this end are the ESR blood-cell count bone marrow examination serum protein electrophoresis, liver and renal function tests and chest radiography The presence of ECG-anomalies may indicate cardiac amyloid deposits Biopsy specimens should be taken from either the oral or preferably the rectal mucosa (Gafni &

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CUFF PRESSURE AND MICROVASCULAR OCCLUSION IN THE TRACHEAL MUCOSA

An Intravital Microscopic Study in the Rabbit

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(Received November 24 1978)

Abstract A method is described for the intravital microscopic observations of rabbit tracheal mucosa microcirculation during compression with thin transparent high-volume cuff The cuff pressure (CP) required to cause complete ischemia in the mucosa over the cartilages was measured and correlation was found to the mean arterial blood pressure (MAP) Ischemia was not observed below CP–MAP ratios of 0 40 for untreated animals or below 0 44 for animals where the MAP had been elevated by adrenalin However marked reduction of the microvascular blood flow was present at lower CP–MAP ratios 0 –0 3 which at MAP of 75 mmHg corresponds to cuff pressures of 15–20 mmHg It is therefore advocated that endotracheal cuff pressures are kept below these values to avoid ischemic tissue injury

It is generally accepted that a major cause of tracheal mucosal damage after endotracheal intubation is the ischemia resulting from the pressure which the cuff exerts on the tracheal wall. To avoid or minimize such trauma the so-called low pressure–high volume cuff has been introduced One of the advantages with this type of cuff is the possibility it affords to control the pressure applied on the tracheal wall It is not known exactly however how such a cuff influences the tracheal mucosa microcirculation in different parts of the wall or at what cuff pressures ischemia occurs.

The aim of the present study was to make observations of the tracheal mucosa microcirculation when subjected to pressure from a very thin and pliable high volume cuff and also to find out whether there is any correlation between the cuff pressure level at which ischemia occurs and the arterial blood pressure

This paper describes a method developed for direct microscopic observation of the tracheal mucosa microcirculation in rabbits using a transparent cuff The cuff pressures required to cause ischemia have been measured and subjected to statistical analysis

MATERIAL AND METHODS

Twelve domestic rabbits weighing 2.5–4 kg were used Anesthesia was induced by intramuscular injection of Hypnorm® Vet (Leo Sweden) 0 7 ml/kg b wt and then maintained by repeated i.m. injections

The trachea was exposed by careful dissection and two ventral holes were made one distal to ensure free airways and one (8×4 mm) just below the larynx for observations as described in detail in a previous report (Stenqvist et al 1978) The animals were breathing spontaneously and the body temperature was kept at 37°C by heating blanket. The mean arterial blood pressure (MAP) was monitored with a mercury manometer connected to a catheter in a mid-ear artery

During the experiments the animals were kept leaning slightly sidewards so that the microcirculation on the lateral wall of the trachea could be observed The tracheal wall was trans-illuminated with a 24 V 250 W halogen lamp the light being transmitted through a flexible fibre-optics bundle and a plexiglass rod with a 45° mirrored bevel at the end. Observations were made with a Leitz stereo

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Table 1 *Cuff pressure (CP) causing complete ischemia over cartilages related to mean arterial blood pressure (MAP)*

Expt no	CP mmHg Aver age	CP mmHg Range	MAP mmHg	CP/MAP Aver age	CP/MAP Range
1	33.3	30–35	75	0.44	0.40–0.47
	52.5	50–55	80	0.66	0.63–0.69
3	55.8	40–60	80	0.70	0.50–0.75
4	34	30–35	70	0.49	0.43–0.50
5	34	30–40	75	0.46	0.40–0.53
6	38.3	35–45	70	0.55	0.50–0.64
7	35.8	35–40	75	0.48	0.46–0.53
	54	45–60	100*	0.54	0.45–0.60
8	35.8	35–40	70	0.51	0.50–0.57
	50.0	40–55	90*	0.56	0.44–0.61
9	33.3	30–35	65	0.51	0.46–0.54
	65.0	60–70	115*	0.56	0.57–0.61
10	42.5	35–50	75	0.57	0.47–0.67
	67.5	60–75	100*	0.68	0.60–0.75
11	39.1	35–45	70	0.56	0.50–0.64
	45.0	40–50	90*	0.50	0.44–0.56
1	42.5	30–50	75	0.57	0.40–0.67
	78.3	75–85	125	0.6[illegible]	0.60–0.68

Blood pressure elevated by adrenalin

0.8221 $p<0.001$ for the animals treated with adrenalin) as calculated with the Spearman rank correlation test. Complete ischemia over the cartilages was not seen below a CP–MAP ratio of 0.40 for untreated animals or below 0.4 for adrenalin treated animals.

While complete ischemia over the cartilages was obtained at fairly low cuff pressures the microcirculation between the cartilages was usually little affected at these pressure levels. Thus a cuff pressure above the estimated systolic blood pressure often had to be applied to achieve complete circulatory standstill in these regions.

DISCUSSION

The study shows that for the microcirculation over the cartilages there is a correlation between the cuff pressure (CP) causing complete mucosal ischemia and the mean arterial blood pressure (MAP). This correlation was consistent also when the blood pressure was

To obtain circulatory standstill between the cartilages on the other hand usually very high cuff pressures which could not be related to the blood pressure had to be used. These different cuff effects over and between the cartilages agree with previous observations by Nordin et al (1977) and Galoob et al (1977) although it should be remarked that apparently other types and qualities of cuffs were used in those studies.

The explanation of the phenomenon ought to be found in the fact that even if a very thin and pliable cuff is used which closely drapes the entire mucosa instead of bridging the intercartilaginous spaces as a less pliable cuff would the mucosa between the cartilages will not be compressed but merely pushed laterally as there is little counteracting force from the tissue around the trachea. Thus in respect to influences on the intercartilaginous microcirculation there will be little difference between a high volume–low pressure cuff and a low volume–high pressure cuff.

In this study we have established that total ischemia will occur in the tracheal mucosa over the cartilages at cuff pressures of about 40% of the MAP. Our observations indicate however that significant disturbances in the nutritional blood flow of the mucosa occur at CP–MAP ratios which are well below 0.40. Thus we observed that the blood flow over the cartilages was usually much reduced already at cuff pressures of 15–20 mmHg which in the typical case of an MAP of 75 mmHg corresponds to a CP–MAP ratio of 0.2–0.3. Against this background we strongly support the recommendation previously put forward by Nordin et al (1977) that the pressure in endotracheal cuffs should not be raised above about 20 mmHg, in order to minimize the risk of ischemic tissue injury. Furthermore since it is the tissue overlying the cartilages which is most compressed and also first damaged during cuffing (Cooper & Grillo 1969 Hilding 1971) it is *important that the* length of the high volume–low pressure cuffs should be kept as short as possible so as to

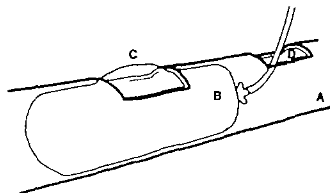


Fig 1 Schematic drawing of trachea with inflated cuff *A* Tracheal wall *B* cuff with tubing to pressure gauge *C* observation hole *D* respiratory opening.

microscope (Model RS) using magnifications ×12.5 ×25 and ×50

Cuffs were made from transparent very pliable polyethylene sheets 0 011 mm thick which were welded into cylinders about 3 cm long and with diameters varying between 5 and 10 mm to fit the various sizes of the tracheas The cuff diameter was always selected to be slightly larger than the inner diameter of the trachea in order to simulate a low pressure–high volume cuff Further no pressure would be required to stretch the cuff ascertaining that when the cuff was inflated the same pressure as in the cuff could be assumed to act on the tracheal wall

The cuff was connected to a polyethylene tube (PK 240 Kifa) which was brought into the trachea through the observation hole and then out through the distal respiratory opening until the cuff was appropriately positioned in the observation area (Fig 1) The cuff was inflated from a calibrated airdriven pressure gauge self-compensating for leakages in the system To avoid fogging of the cuff a continuous air flow was allowed through the cuff by a small leak at the end

Test procedure

Before the cuff was placed in the trachea the microcirculation was observed for about 30 minutes to check that there were no major disturbances of the microcirculation After the cuff had been placed in the trachea a clearly visible area over one cartilage and its adjacent intercartilaginous spaces was selected for observation The cuff pressure was then increased stepwise by 5 mmHg at a time from 0–125 mmHg each pressure level being held for about 10 seconds This procedure was repeated six times at about one minute intervals in each animal The experiments were performed in such a way that the person making the microscopic observations could not know the actual cuff pressure The point of ischemia was defined as the state when no circulation could be observed in any vessel over the cartilages

In 6 animals the blood pressure was elevated by intravenous injection of adrenalin (0.3–0 7 ml) whereafter the same procedure as that described above was repeated

RESULTS

The mean arterial blood pressures (MAP) and the cuff pressures (CP) which cause complete ischemia over the cartilages are presented in Table I and Fig 2 The correlation between CP and MAP is highly significant (r=0 4651 p<0 001 for the untreated animals and r=

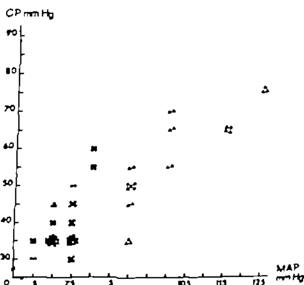


Fig 2 Plots of the cuff pressures (*CP*) causing complete ischemia over the cartilages at different mean arterial pressures (*MAP*) ● = untreated animals ▲ = blood pressure elevated by adrenalin

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STUDIES OF THE EFFECT OF PERORAL FENYLPROPANOLAMIN ON THE FUNCTIONAL SIZE OF THE HUMAN MAXILLARY OSTIUM

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(Received January 8 1979)

Summary The effect of peroral fenylpropanolamin on the functional size of the human maxillary ostium was studied in 20 patients suffering from acute rhino-sinusitis. The size of the maxillary ostium was measured by manometric procedure over -hour period. During the introduction of known airflow into the sinus for short while the pressure increase was compared with nomogram obtained by model experiments The introduction of the cannulas into the maxillary sinus caused swelling of the mucosa in the ostium in the placebo group and initially also in the group receiving fenylpropanolamin, but, after 60 minutes, the latter group had mean functional ostial size greater than the initial one. These differences were not statistically significant This seems to be the first objective study of the size of the ostia in the human paranasal sinuses during treatment with peroral decongestant

The normal maxillary ostium in man is a tubular canal connecting the maxillary sinus to the middle meatus of the nasal cavity The average diameter of this canal is 2.5 mm corresponding to a cross-section area of 5 mm² (Aust & Drettner 1974) and has an average length of 6 mm In some humans however there are one or more accessory ostia present.

The ostial canal(s) is lined with respiratory ciliated epithelium This mucosal membrane is well vasculated with a rich network of veins The mucosa of the nose the paranasal sinuses and the ostia have a pronounced tendency to become swollen when irritated by mechanical chemical infectious or allergenic agents, narrowing the passage through the nose and the ostia, thus hindering both the ventilation and drainage of the paranasal sinuses Treatment with the purpose of reducing mucosal swelling in the upper respiratory tract, for example in the ostia is consequently given in various diseases such as acute rhino-sinusitis Since nasal drops containing decongestants are not recommended for more than a period of 10 days and as they sometimes hardly penetrate to the ostia, perorally administrated decongestants are widely used The effect on the nasal patency of such peroral treatment alone (Roth et al., 1977) or in combination with antihistamine (Aschan 1974) has been objectively verified but so far there has been no objective assessment concerning the effect of peroral decongestants on the patency of the ostia in the paranasal sinuses in man. Fenylpropanolamin is one commonly used peroral sympatomimeticum either alone or in combination with antihistamine It has the same decongestive effect as ephedrine but has less effect on the central nervous system. The aim of this work is to investigate experimentally the effect of fenylpropanolamin on the size of the maxillary ostium in living man with rhinosinusitis

MATERIAL

Twenty patients 8 women and 12 men aged 16–56 years all showing symptoms of acute rhino-sinusitis were investigated concerning the size of the maxillary ostium before and after medication with either fenylpropanolamin or placebo Only those persons where the maxillary ostium was patent during ordinary respiration were included This evaluation was performed by simple manometric measurement of the maxillary sinus during nasal breathing (Drettner 1965)

interfere with as few cartilages as possible a large cuff volume should rather be obtained by a large diameter

ZUSAMMENFASSUNG

Es wurde eine Methode für vitalmikroskopsche Studien von der terminalen Strombahn der Schleimhaut der Kaninchentrachea während Kompression mit einer großen und sehr dünnwandigen Schlauchmanschette beschrieben Es liegt ein Zusammenhang zwischen dem Druck der Schlauchmanschette (cuff pressure CP) der totale Ischämie und Trachealknorpeln produziert und dem Blutdruck (mean arterial blood pressure MAP) vor Totale Ischämie wurde nie unter einer CP–MAP Quote von 0 40 beobachtet. Deutliche Störungen in der Zirkulation der terminalen Strombahn wurde jedoch an einer CP–MAP-Quote von 0,–0,3 (entsprechend einer CP von 15–20 mmHg an einer *MAP* von 75 mmHg) beobachtet. Es wurde deshalb vorgeschlagen daß der Druck einer Schlauchmanschette niemals dieses Niveau erreichen soll um ischämische Schleimhautschädigungen in der Trachea zu vermeiden

ACKNOWLEDGEMENT

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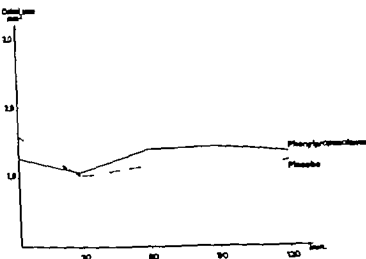


Fig 2 The functional size of the maxillary ostium (expressed as cross-sectional area in mm²) in 20 patients with acute rhino-sinusitis Double-blind test with administration of 100 mg fenylpropanolamin or placebo. The results are given as the mean values

maxillary ostium was measured after 30 60 90 and 120 minutes During these ? hours the patient remained in the same position on the couch

To get an idea of the long-term effect of the drug the patients were given 100 mg fenylpropanolamin twice daily or placebo twice daily for 7 days in a double-blind procedure The patients were asked to complete a form each day in which they recorded their subjective experience of any effect on their symptoms from the administered medicine Furthermore the patients were asked to describe side effects if any Together with the drugs used in the experiments the patients were given antibiotics (V-penicillin 0.8 mg ×)

RESULTS

Short-term observation

The mean value of the functional size of the ostium before the administration of the tablets was somewhat bigger in the placebo group than in that receiving fenylpropanolamin (Fig.) The mean ostial size decreased initially in the placebo group and did not recover the original val e during the -hour observation period. The group receiving fenylpropanolamin had a small initial decrease in ostial size but already after 60 minutes the mean ostial size had exceeded the original value and remained at that level during the following hour The dispersion was great and the differences between the two groups were not statistically significant at any of the time points during the observation period

Long-term observation

None of the parameters recorded by the patients concerning symptoms from the nose and paranasal sinuses showed any difference between the two groups during the 7 days of treatment Neither did side effects attributable to the treatment show any differences between the two groups

DISCUSSION

The maxillary ostium and other ostia of paranasal sinuses play an important role in the pathophysiology of sinusitis The mucosal lining of the ostium which swells easily can rapidly alter the ventilation and the drainage of the paranasal sinuses and thus in a short time change the environment for microorganisms dwelling in these cavities It has not earlier been possible in living man to measure changes in the functional size caused by irritation and/or medication

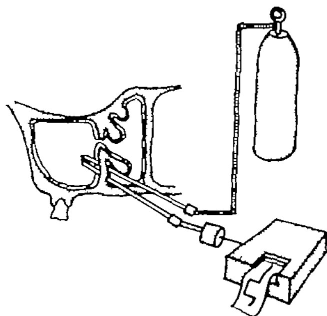


Fig 1 The method for measuring of the functional size of the human maxillary ostium Two cannulas are introduced through the inferior meatus into the maxillary sinus after topical anesthesia without any decongestive effect By pressure recording from each cannula separately during respiration it can be checked that the ostium is patent and that the cannulas are not situated in the mucosa One cannula is then used for introduction of an airstream of known airflow (1 4 or 6 l/min) for a few seconds The other cannula is used for pressure recordings from the sinus The pressure increase is compared with the results of nomograms obtained by measurements n a model with different ostial size

METHODS

The size of the maxillary ostium in living man was investigated according to a previously described method (Aust & Drettner 1974) whereby two cannulas are introduced into the maxillary sinus as shown in Fig 1 Xylocaine spray without any decongestant was used as anesthesia One of the cannulas was connected to a manometer (EMT 31) and a recorder (Mingograph no 34) and the other was connected to a tube with compressed air and a flow meter Model experiments in which the maxillary sinus was replaced by a syringe with a variable opening to simulate the ostium showed that a small airflow introduced into the sinus gave a measurable pressure rise in the investigated antrum related to the ostial size but independent of the antral volume

From the model experiments a nomogram was constructed showing the relationship between airflow antral pressure rise and ostial size From this nomogram the size of the ostium in human experiments could be calculated from the flow of air introduced into the sinus and the resulting antral pressure rise recorded with the manometer and the mingograph connected to the sinus via the cannula.

We could not judge whether the investigated sinus had only one ostium or if there were any accessory ostia present Therefore we called the manometrically measured size of the ostium or ostia 'the functional size of the maxillary ostium

The effect of fenylpropanolamin on the functional size of the maxillary ostium in patients with acute rhino-sinusitis was studied in a double-blind test The patients who were randomly given perorally either fenylpropanolamin or placebo were investigated both regarding short and long term effects

Experimental procedure

After a clinical routine investigation and roentgenography of the maxillary sinuses the patient was placed on a couch in a semi-recumbent position

The experiment started with the introduction of two cannulas through the lower middle nasal meatus into the maxillary sinus The patency of the cannula and the ostium was tested when the patient breathed first quietly and then heavily through the nose The respiratory pressure changes were recorded first from one cannula and then from the other demonstrating that the two cannulas as well as the ostium were patent and thus allowing the manometric method for measuring the ostial size to be used without any risk of air embolism

The initial functional size of the maxillary ostium was measured according to the method described and immediately thereafter 100 mg fenylpropanolamin or placebo was administered perorally The functional size of the

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VASOPRESSIN FOR BLEEDING FROM THE HEAD AND NECK

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(Received March 27 1979)

Abstract Excessive bleeding from the head and neck sometimes presents therapeutic problem Ten patients ith this type of haemorrhage have been treated with continuous vasopressin infusion for 1 3 days. Haemostasis was quickly established in eight cases No serious side effects were noted Infusion of vasopressin has rapid beneficial effect on haemorrhage from branches of the external carotid artery

For the control of serious epistaxis, various types of tamponade and antifibrinolytic agents have been recommended (Petruson 1974) Nasal tamponade is accompanied by inconvenience and discomfort for the patient It is painful to insert prevents normal breathing and may cause local infection. If the tamponade fails, the supplying artery may have to be ligated, e g. the external carotid artery or the internal maxillary artery (Chandler & Serrins 1965)

Because it can be hard to locate the exact point of bleeding and there are multiple anastomoses in the nose one somtimes has to ligate several different vessels (Shaheen 1970)

With bleeding from the tonsils or posttonsillectomy one usually has to ligate vessels in the tonsillar bed To achieve permanent haemostasis the external carotid artery may need to be ligated These measures may lead to increased risk during intubation especially of aspiration of blood into the lungs

Other causes of serious bleeding from the external carotid include facial injuries tumours post-adenoidectomy and dental extraction There is no single simple method of controlling bleeding from the head and neck.

The aim of this investigation was to determine whether vasopressin would be useful in such situations

Vasopressin causes vasoconstriction by acting directly on the smooth muscle of the vessels There is a simultaneous decrease in the coronary blood flow Vasopressin should therefore probably not be used on patients with serious angina pectoris and recent myocardial infarction

Vasopressin has been used locally to reduce bleeding and to prolong the effect of local anesthesia (Klingenstrom et al 1967)

Angiographic and haemodynamic studies on dogs have shown that vasopressin shunts the bloodflow from the external to the internal carotid artery (Nylander 1967 Ericsson 1971) It has not been established whether the same shunt mechanism is present in man

In patients with moderate and mild haemophilia and von Willebrand's disease vasopressin induces a marked rise in autologous Factor VIII which is haemostatically effective providing that adequate plasma concentrations are attained (Mannucci et al 1977)

Synthetic vasopressin has therefore several favourable properties for the treatment of bleeding from the head and neck

MATERIAL AND METHOD

Ten patients with uncontrolled bleeding from branches of the external carotid artery have been treated with continuous infusion of vasopressin in physiological saline Two patients were given 40 IU ornithine-8-vasopressin

In all experiments we found that the functional size of the maxillary ostium was reduced within the first half hour after the introduction of the cannulas into the maxillary sinus This reduction in ostial size was probably a result of mechanical irritation on the nasal and antral mucosa caused by the two cannulas introduced through the lower nasal meatus This reduction persisted in the placebo group during at least 2 hours while the group receiving active medication had a less pronounced and briefer reduction in size changing to a larger ostium than originally after one hour However the differences were too small to be significant

ACKNOWLEDGMENT

This work was supported by the Swedish Medical Research Council (Project 749)

ZUSAMMENFASSUNG

Der Effekt einer oralen Zufuhrung von Fenylpropanolamin an der funktionellen Größe des Kieferhöhlenostiums wurde an 70 Patienten mit akuter Rhinosinusitis studiert Die Größe des Kieferhohlenostiums wurde mit manometrischer Methode unter einer Zweistundenperiode gemessen Unter Einfuhrung eines Luftstromes in die Kieferhöhle während einer kurzen Zeit wurde die Druckerhohung durch eine andere Kanule in der Kieferhohle gemessen und das Resultat mit einem Nomogram verglichen welches man von einem Modellversuch erhielt. Das Einfuhren der Kanule in die Kieferhohle verursachte eine Schleimhautanschwellung im Ostium der Placebogruppe und mutual auch in der Gruppe die Fenylpropanolamin erhielt Nach 60 Minuten hatte die spätere Gruppe einen Durchschnittswert für die funktionelle Ostiumgroße die großer war als die ursprungliche Diese Unterschiede waren doch statistisch nicht signifikant. Dies scheint das erste objektive Studium der Ostiumgroße *in der Kieferhöhle des Menschen* wahrend einer Behandlung mit peroralen abschwellenden Mitteln zu sein

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VASOPRESSIN FOR BLEEDING FROM THE HEAD AND NECK

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From the Department of Ear Nose and Throat Central Hospital Helsingborg Sweden

(Received March 27 1979)

Abstract Excessive bleeding from the head and neck sometimes presents a therapeutic problem Ten patients with this type of haemorrhage have been treated with continuous vasopressin infusion for 1 3 days. Haemostasis was quickly established in eight cases. No serious side effects were noted Infusion of vasopressin has a rapid, beneficial effect on haemorrhage from branches of the external carotid artery

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ZUSAMMENFASSUNG

Der Effekt einer oralen Zuführung von Fenylpropanolamin an der funktionellen Größe des Kieferhöhlenostiums wurde an 20 Patienten mit akuter Rhinosinusitis studiert. Die Größe des Kieferhöhlenostiums wurde mit manometrischer Methode unter einer Zweistundenperiode gemessen Unter Einführung eines Luftstromes in die Kieferhöhle während einer kurzen Zeit wurde die Druckerhöhung durch eine andere Kanüle der Kieferhöhle gemessen und das Resultat mit einem Nomogram verglichen welches man von einem Modellversuch erhielt Das Einführen der Kanüle in die Kieferhöhle verursachte eine Schleimhautanschwellung im Ostium der Placebogruppe und initial auch in der Gruppe die Fenylpropanolamin erhielt. Nach 60 Minuten hatte die spätere Gruppe einen Durchschnittswert für die funktionelle Ostiumgroße die größer war als die ursprüngliche. Diese Unterschiede waren doch statistisch nicht signifikant Dies scheint das erste objektive Studium der Ostiumgröße in der Kieferhöhle des Menschen während einer Behandlung mit peroralen abschwellenden Mitteln zu sein

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the head and neck (Klingenström et al 1976 Nylander 1967 Ericsson 1971 Mannucci et al. 1977).

In the present series haemorrhage was controlled in 8 of 10 patients. The lack of effect in one patient (no 6) might have been because the bleeding originated from the ethmoidal vessels. Patient no 7 stopped bleeding relatively late The patient suffered from arteriosclerosis which might have impaired the ability of the vessels to constrict. The investigation has shown that vasopressin infusion is a simple and effective method of achieving haemostasis. The treatment can be started promptly and can be given concurrently with other therapy

ZUSAMMENFASSUNG

Blutungen im Hals-Nasen-Bereich sind zuweilen schwer zu beherrschen. Gewöhnlicherweise ist der Blutungsherd eine Art der A carotis externa Wir haben versucht, solche Blutungen mit kontinuierlicher Infusion von "Vasopressin" zu behandeln, wobei gute Resultate in acht von zehn Fällen erreicht wurden. Die Infusion von Vasopressin erwies sich also sowohl einfach als auch effektive Behandlungsmethode.

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Table I *Results of vasopressin infusion for control of haemorrhage*

Patient no	Age/sex	Diagnosis	Time until bleeding stopped (min)	Duration of treatment (h)	Result
1	17/♂	Posterior epistaxis	5	48	Effective
2	26/♂	Post tonsillectomy bleeding	10	48	Effective
3	28/♀	Post-dental bleeding	5	4	Effective
4	52/♂	Posterior epistaxis	10	7	Effective but discontinued
5	34/♂	Posterior epistaxis	5	24	Effective
6	44/♂	Posterior epistaxis	45	64	Ineffective
7	80/♀	Posterior epistaxis	45	45	Effective
8	41/♂	Posterior epistaxis	5	37	Effective
9	10/♂	Post tonsillectomy bleeding	15	4	Effective
10	54/♀	Posterior epistaxis	10	30	Effective

(POR-8 Sandoz) on the first day and 20 IU on subsequent days The remaining 8 patients received between 60 and 120 IU lysine–vasopressin (Postacton Ferring) daily Initially the infusion rate was maximal until the patients became pale and complained of abdominal pain The infusion rate was then slowed but kept as fast as the patient could tolerate

Occasionally haemorrhages developed in association with coughing or exertion The infusion rate was then increased The treatment was started during active bleeding in every case continued for a maximum of 3 days and terminated promptly

Conventional therapeutic regimens had been used unsuccessfully in 7 of the patients for between one hour and 10 days prior to the administration of vasopressin These regimes included compression tamponade and antifibrinolytic drugs In the 2 post tonsillectomy patients and one patient with posterior epistaxis vasopressin was used as a first line of treatment

Every patient was given antifibrinolytic agents One patient also received vitamin K because alcoholic induced liver disease was suspected

RESULTS

One of the 10 patients in the study (no 6) had repeated epistaxis in spite of the infusion (Table I) Another patient (no 7) did not stop bleeding for 45 min The same patient developed fresh bleeding 15 days after the treatment was completed The remaining 8 patients had a good response but one patient (no 4) subsequently had to cease the treatment because of delirium tremens Two patients were chronic alcoholics (nos 4 and 6)

Side effects noted which restricted the rate of infusion were colicky abdominal pain and diarrhoea When the infusion was slowed these effects were no longer a problem The antidiuretic effect of vasopressin usually resulted in a compensatory polyuria after the treatment Two patients (nos 8 and 7) developed pleural effusions which resorbed spontaneously and quickly

DISCUSSION

Haemorrhage from the head and neck may be difficult to control Usually the bleeding originates from branches of the external carotid artery rarely is the bleeding from the ethmoidal arteries which arise from the internal carotid artery It would be a significant advance if a simple method could achieve haemostasis without the need for tamponade or ligation Previous investigations have suggested that vasopressin has several different effects that would be useful for haemostasis of



Fig. 1. Section from the removed parotid gland in case A, showing squamous cell carcinoma surrounded by connective tissue with Thorotrast granules (H and E, ×150).

operation was performed on December 19 1974. Biopsies now showed definite malignancy and total parotidectomy was done together with radical neck dissection on the right. Recurrence was diagnosed by exploratory biopsies in April 1975 and the patient died in May 1977. Autopsy was not performed.

Case B

A 49-year-old woman was admitted in February 1976 with a tumour in the left parotid gland. In 1931 at the age of 5 the patient had experienced relapsing swelling of the left parotid gland. A diagnosis of chronic parotitis was suggested and sialography was carried out using ½ ml Thorotrast®. In 194 an operation of unknown nature had been performed on the gland. From 1966 she developed sensations in the left parotid region and a small tumour in the gland. However the tumour remained unchanged until 1975 when partial left-sided facial nerve paralysis of peripheral type and further growth of tumour were noticed.

On admission a firm immobile indolent cystic tumour was found. It was ill-defined and about the size of a hazel nut. Clinically there was a strong suspicion of malignancy and a biopsy taken on February 27 showed squamous cell carcinoma. On March 15 1976 the patient underwent total parotidectomy which was not entirely radical however. In June 1978 after a period with pain surgical exploration revealed a large local recurrence and the patient was referred for radiation and cancer chemotherapy.

Pathology

Microscopic investigation of paraffin sections routinely stained with hematoxylin-eosin and by the method of van Gieson–Hansen showed squamous cell carcinomas, a type of tumour rarely found in the parotid gland. As no tu

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CARCINOMA OF THE PAROTID GLAND FOLLOWING SIALOGRAPHY WITH THOROTRAST®

Report of Two Cases

Maja Nielsen Reidar Albrechtsen Niels Jon Johnsen and Jakob Visfeldt

From the Departments of Pathology and ENT Rigshospitalet Copenhagen Denmark

(Received February 5 1979)

Abstract Two cases are presented of malignant tumour of the parotid gland following sialography with Thorotrast® 28 and 45 years previously Both cases were histologically established as squamous cell carcinoma and the presence of Thorotrast® in the tumours was confirmed by autohistoradiography It is suggested that the tumours may have developed from metaplastic ductal epithelium after many years of exposure to the alpha radiation from Thorotrast deposits in the gland

Colloidal thorium dioxide was introduced as a radiological contrast medium about 1928 (Mitchell 1973) The most important preparation Thorotrast® was widely used in Denmark during the years 1935 to 1947 (Faber 1973) It was used mainly for cerebral angiography but also (though rarely) for sialography It is well known that after intravascular injection Thorotrast® remains in the body as deposits in the liver spleen bone marrow and other organs of the reticuloendothelial system Serious late toxic effects ascribed to Thorotrast developing many years after its administration have been established by studies of comprehensive Thorotrast series (Faber 1973) It appeared that Thorotrast carriers had an increased incidence of liver tumours liver cirrhosis leukaemias and aplastic anaemias (e g Faber 1967 1973 Visfeldt & Poulsen 1972)

We present here 2 cases of carcinoma of the parotid gland which could be regarded as late effects of Thorotrast® used in sialography So far these cases both squamous cell carcinomas are the only ones published

CASE REPORTS

Case A

A 59-year-old woman was admitted in January 1974 with a tumour in the right parotid gland The tumour had been present for many years without significant growth During the month before admission she developed severe pain around the ear and slowly progressing facial paralysis Her past history included sialography of the right parotid gland performed in 1946

Examination revealed a firm indolent mass the size of a hazel nut in the right parotid region It was ill defined and appeared to be deeply fixed There was partial right facial nerve paralysis of the peripheral type Fine needle biopsy yielded only peripheral blood Malignant transformation of a pleomorphic adenoma in the parotid gland was suspected and the first operation was performed on January 10 1974 The gland was infiltrated by dense tumour like tissue so that the main trunk of the facial nerve could not be isolated. Two biopsies were sent for frozen section and the diagnosis was connective tissue with conspicuous pigment without evidence of malignancy The operation was therefore discontinued However the facial nerve paralysis progressed slowly and in 6 months had become total At clinical examination no tumour growth was demonstrable until 10 months after the first operation At that time a rapidly growing tumour developed in the region A new



Fig 1 Section from the removed parotid gland in case A, showing squamous cell carcinoma surrounded by connective tissue with Thorotrast granules (H and E. 150)

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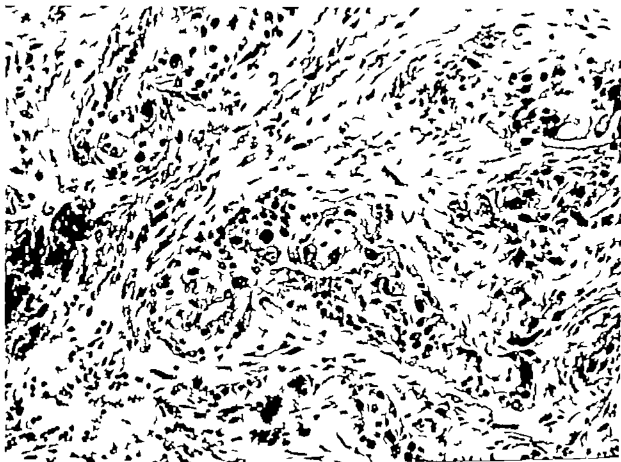


Fig 2 Section from the tumour in case B showing squamous cell carcinoma with Thorotrast® deposits in the connective tissue surrounding nests of tumour cells (H and E ×250)

mour was present in other sites the tumours are considered primary parotid neoplasms

Case A

Biopsies from the parotid region at the first exploration showed no remaining normal gland structures—only dense connective tissue with thick collagen bundles which in many places showed hyaline transformation Inconspicuous vessels were found without thrombosis or inflammation The collagen bundles were interspersed with brown pigment both extracellularly and in the cytoplasm of macrophages Stainings of the pigment for iron and melanin proved negative There was no evidence of malignancy The diagnosis was connective tissue with pigment presumably Thorotrast[2] and this was confirmed by autohistoradiography

Sections from the parotid gland removed 11 months later showed poorly differentiated squamous cell carcinoma with a low degree of keratinization Thorotrast® deposits were localized in the dense connective tissue in the immediate vicinity of the tumour cell infiltrates (Fig 1) There was no tubule formation Autohistoradiography was performed EM-examination was not done

Case B

Histological examination of the first biopsy material showed no preserved parotid gland structures—only connective tissue infiltrated by well differentiated squamous cell carcinoma Abundant brown pigment compatible with Thorotrast® was found in the vicinity of the tumour tissue extracellularly as well as in the cytoplasm of the macrophages Stainings of the pigment for iron and melanin were negative

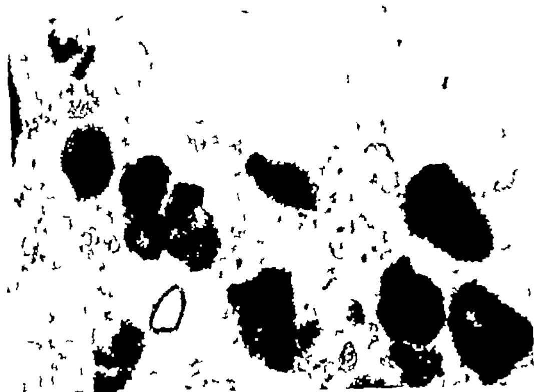


Fig. 3. Aggregates of electron-dense granular material of varying size representing Thorotrast deposits (×1000)

When removed the parotid gland appeared firm, scirrhous and pale. Histological sections showed that the whole gland had been replaced with hyalinized connective tissue infiltrated by a well differentiated squamous cell carcinoma showing varying degrees of keratinization. Areas of focal necrosis were present. There were no duct like structures. In this case too Thorotrast[2] deposits were observed in the collagen bundles surrounding the nests of tumour cells (Fig. 2). Some vessels showed arteritis obliterans.

The tumour tissue was divided into × ×3 mm pieces, fixed in 5% glutaraldehyde in isotonic cacodylate buffer (pH 7.5), post-fixed in osmium tetroxide, dehydrated in alcohol and embedded in Vestopal W. Ultrathin sections were made from selected areas and stained with magnesium uranyl acetate, lead citrate and examined in a Zeiss 10 A. Autohistoradiography was performed.

Electron microscopy revealed that most of the tissue was acellular and composed of a collagenous stroma in which aggregates of an electron-dense granular material of varying size could be seen representing characteristic Thorotrast deposits (Fig. 3). Intermingled between these deposits remaining cytoplasmic organelles were found. Thorotrast deposits were also observed in areas surrounding epithelial tumour like cells (Fig. 4).

Quantitative measurements of the content of thorium or the intensity of radiation were not attempted.

DISCUSSION

The two case reports indicate that Thorotrast[2] when retained in the parotid gland after



Fig 4 Thorotrast® deposits found in areas surrounding an epithelial tumour-like cell (× 15 000)

local administration by sialography is capable of inducing fibrosis and after years malignant transformation

The only earlier report of malignant tumour developing after sialography with Thorotrast® is case A from our hospital published by Johnsen et al in 1976 Verhaeghe et al published a case (1970) of thorotrastoma of the parotid gland with palsy of the facial nerve observed 34 years after parotid sialography Total parotidectomy disclosed fibrosis but no evidence of malignancy

To produce satisfactory evidence of a relationship between Thorotrast® and the malignant tumours as emphasized by Dahlgren (1967) the following criteria should be fulfilled

1) Thorotrast deposits must be present in the immediate vicinity of the primary tumour

2) the latent period must be sufficient long

3) the amount of Thorotrast and consequently the radiation dose must be sufficiently high

The first two criteria were fulfilled in the present cases Thorotrast deposits were demonstrated in the immediate vicinity of the tumour tissue (Figs 1 and 2) and the latent period after exposure was long viz. 28 and 45 years The mean latent period for many solid tumours induced by radiation is about 25 years (United Nations 1977) The third criterion demanding a reasonable amount of contrast medium and thereby of radiation is more difficult to evaluate but was probably fulfilled when considering the modest amount of affected tissue In sialography ½–1 ml of contrast medium is used

Thorotrast was used and about the same volume was probably applied in case A. However only a small fraction of this amount can be assumed to have been retained within the parotid tissue.

The content of ^{232}Th in Thorotrast® is about 25 nCi/ml. Thorium-232 has a half life of 1.4× 10^{10} years and emits alpha particles with an energy of about 4 MeV (United Nations 1977). It might be of significance to the carcinogenic effect that alpha radiation can be absorbed in necrotic cells. It has also been discussed whether thorium dioxide besides its radioactivity might possess specific chemical properties contributing to its carcinogenic action (United Nations 1977).

The histological picture in both cases showed squamous cell carcinoma, which is rare in the salivary glands. However the epithelium in the duct system of the parotid gland often shows squamous cell metaplasia, especially when stones and chronic inflammation are present. The development of squamous cell carcinoma induced by Thorotrast® radiation might well have taken place from metaplastic ductal epithelium.

ZUSAMMENFASSUNG

Zwei Fälle von bösartigen Geschwülsten in der glandula parotis nach Sialografi mit Thorotrast 28 und 45 Jahren früher wurden beschrieben. Die Beiden Fällen waren histologisch carcinoma planocellulare und das Vorkommen von Thorotrast® in den Geschwülsten wurde von Autohistoradiografi bestätigt. Es ist vorgeschlagen, dass die Geschwülste sich möglicherweise von metaplastisch ducto-epithelium nach vielen Jahren von Exposition der alfa-Strahlen Thorotrast in der Druse entwickelt haben.

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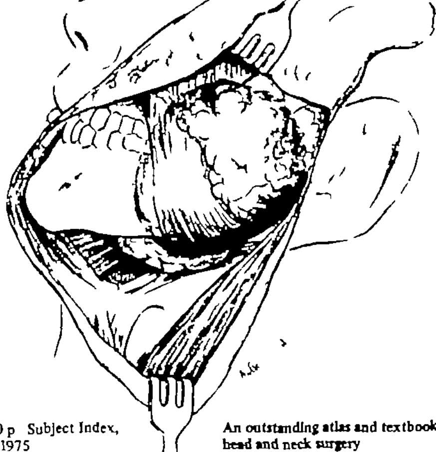
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Sweden: G Dohlman, H. Engström, G Herberts, L. Holmgren, Hj Koch, G Lidén, N Lundgren, A. Sjöberg
Switzerland: F Escher E. Lüscher, A. Montandon, C. R. Pfaltz, L. Rüedi, J P Taillens, A. Weder
USA L. F Boies, J E. Bordley T Cody D A. Hilding, H. P House, G. Kelemen, J R. Lindsay M. M. Paparella, H. F Schuknecht, B. H. Senturia, G E. Shambaugh, Jr., F A. Sooy W P Work
USSR. M. Kchodiakov S Khechinashvili, N. A. Preobrashensky

Conferences and Meetings

1980 Jan. 14–18. XVth Annual Otologic Surgery Course will be held at the Ear Research Institute in Los Angeles, USA. Further information. F H. Linthicum, Jr M.D., Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980 Febr 4–March 15 Otolaryngology Pediatric Course to be held in Barcelona, Spain. Further information. Catedra de O.R.L., Facultad de Medicina, Calle Casanova 143, Barcelona-36, Spain.

1980, March, April, June, October Two-weeks Temporal Bone Surgical Dissection Courses will be held at the Ear Research Institute, Los Angeles, USA. Further information Antonio De La Cruz, M.D., Director Temporal Bone Surgical Dissection Course, Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980, March 2–8 Departments of Otolaryngology of University of Toronto and University of Pittsburgh announce Winter Meeting 1980—Mont Trembland Lodge. Addr Dr William Crysdale, Suite 6118, 555 University Avenue, Toronto, Canada M5G 1X8.

1980, March 3–7 Six Shambaugh International Workshop on Otomicrosurgery and Third Shea Fluctuant Hearing Loss Symposium. For further information Write Mr Herbert Carroll, National Hearing Association, 1010 Jorie Boulevard, Suite 308, Oak Brook, IL 60521 USA.

1980, April 8–12 First World Biomaterials Congress will be held in Baden near Vienna. Information World Biomaterials Congress Secretariat, Mrs E. Maurer Medical Academy of Vienna, Alser Strasse 4 A 1090 Vienna, Austria.

1980, April 13–19 Post Graduate Course in Ear Surgery to be held in Nijmegen, the Netherlands. Further details. Professor Dr W F B. Brinkmann, Department of Otolaryngology Philips van Leydenlaan 15 6500 HB Nijmegen, the Netherlands.

1980 April 20–26. The celebrate the Centenary of the "Revue de Laryngologie, Otologie, Rhinologie" and of the "Ecole d'Oto-Rhino-Laryngologie" scientific and social manifestations are planned in Bordeaux, I. International Postgraduate Course and II. International Symposium. Further information for Secrétariat du Centenaire, 114, avenue d'Arès, 33 000 Bordeaux, France.